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THE ACTION OF DRUGS ON THE SALIVARY SECRETION

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In the course of some work upon expectorants questions arose whose answer it seemed might be suggested by experiments on the salivary gland. These as a result were undertaken and as they seemed of some interest are reported in this paper.

Drugs can act upon the salivary mechanism obviously in several ways, viz. reflexly through the center, directly upon the center, directly upon nerve endings in the glands, directly upon the gland cells without being excreted in quantity by them (physostygmine) or if a selective excretion be presupposed it might be suggested that the presence of certain substances in the blood stream would bring about a salivary flow owing to the necessity for their excretion. A study of the influence of drugs upon the salivary secretion thus necessitates a study of the activities of the salivary center. A nucleus salivatorius was described in 1902 by Kohnstamm. (7) It consists of a group of cells in the upper part of the pons close to the origin of the Nervus Intermedius Wrisbergi; from it arose the fibers of the Chorda Tympani. Whether the nerves to the other salivary glands arise from the same nucleus is not known. The salivary center is very readily depressed by the action of anesthetics. As may be seen from the protocols of experiments 27 and 28 morphine even in small doses depresses the center. If ether or chloroform is pushed to such an extent as to seriously lower the blood pressure or even temporarily stop natural respiration the salivary center will be found inactive often for a prolonged period of time, one or two hours. Strychnine seems to serve in these cases to aid slightly in restoring the function as in experiment 20. Urethane must also be used cauti-

ously. High pithing or intracerebral magnesium chloride both depressed the salivary center in cases where the respiratory center continued to act. It may be readily understood that great difficulty was experienced in obtaining results of the reflex or central actions of drugs. In every experiment the center cannot have been normal in activity when the drugs were tried and if the action of any one either upon the center or sensory periphery be slight it is possible that it has been overlooked. More than one experiment was tried with every drug-stuff though often but a typical one is given in the protocols.

The afferent paths to the center seem very wide. In Jappelli's (2) experiments, cortical stimulation resulting in epilepsy brought about secretion and a mental effect on the salivary flow is well known. Stimulation of almost any sensory nerve will bring about secretion if the center be active (see experiments 27 and 28 for the crural nerve and experiments 22, 27 and 28 for the lingual). The lingual nerve seems to bring about secretion most readily and in largest amount, experiment 28. Secretion begins after a very short latent period, in experiment 28 three seconds, and often outlasts the stimulation, for example in experiment 22, a stimulus of three minutes duration with a strength of current which was easily borne on the tongue caused a flow of 20 drops beginning in 20 seconds and outlasting the stimulus by at least 150 seconds. In the experiments reported by Jappelli (3), the center must have been depressed. Electrical stimulus does not seem to be very adequate, as the application of ether to the tongue brings about a much more rapid and more certain response, *i.e.*, it will produce a flow when lingual stimulation even with a strong current fails to do so. As ether was frequently employed to test the efficiency of the center (experiment 28), I interrupt to describe how it was used. Two canulæ were tied into the trachea the lower one serving for respiration while through the upper a blast of air passing through ether in a bottle and so saturated with its vapor could be directed against the nerve endings in the mouth. The blast or blasts containing ether were always followed by blasts of air to remove the ether from the mouth and larynx. With this method a roughly quantitative stimulation with ether could be given. Ether

serves as a stimulus not only when allowed to act upon endings in the mouth but also when passed into the trachea (experiment 19).

The efferent secretory paths are two in number, the chorda and the sympathetic. A rough comparison between the amount of stimulus coming over these two routes was obtained by cutting the chorda on one side and the sympathetic on the other (experiment 28). The side with the chorda intact produced 20 to 40 times as much saliva due to reflex stimulation as did that with the sympathetic alone intact. The route from the center in cortical epilepsy is stated by Jappelli (2) to be the chorda. In consequence of the relatively small reflex secretion from a gland whose chorda was cut it was not considered necessary to cut the sympathetic in all cases in order to detect whether the action of a drug-stuff was central or peripheral in the gland.

Variations in temperature have a slight effect upon the flow of saliva and Jappelli describes an interesting case of the effect of heat reported by Parfenow. In experiment 5 warm saline produced a very much more marked flow of saliva than did the same amount of a solution at body temperature. This suggests an action due to the heat of the solution. Blood-pressure too has some effect upon the flow of secretion; this point was, however, not examined in detail but care was taken to work under pressures which were as normal as could be achieved.

It has been suggested that certain substances excreted by the salivary glands owing to their excretions stimulate the glands to an increased production of saliva. It has been recently shown by DeSouza (1) that sulphocyanates long considered a specific secretion of the salivary glands are only excreted when present in the blood stream and always occur in the saliva in smaller quantities than in the blood and are also excreted by the kidneys, liver and pancreas. As shown in protocols 28 and 19, their presence in the blood does not serve to produce a flow of saliva. Observations on nitrites, as in experiment 29, show also that they do not act as a specific stimulus. They are also not usually present in dog saliva (the excretion of nitrites will form the subject of a subsequent communication).

The method employed in this series of experiments consisted

in anaesthetizing the animals, dogs were almost exclusively used, with a mixture of ether and chloroform. The operation consisted in laying bare both submaxillary ducts and inserting canulæ into them. Canulæ were placed in the femoral vein and in the femoral artery for injection and the recording of the blood-pressure. In those cases in which the chorda was cut great care was taken that all accessory branches from the lingual to the gland were also destroyed. Urethane was usually given intravenously in a quantity sufficient to keep the animal quiet during the period of experimentation.

Potassium iodide has often been stated to increase the salivary flow and indeed increased salivation during the administration of iodides forms one of the symptoms of "Iodism" though the appearance of this symptom is by no means constant. It is well known that when iodides are given *per os* they may be found in the saliva within a few minutes. Experiment 1 shows conclusively that small doses of therapeutic size do not directly cause the gland to secrete, the extra drop secreted on the left side in this case as compared with the one drop previously secreted was undoubtedly due reflexly to the slight struggles of the animal which also brought about a spurt of five or six drops on the right side. The salivary secretion for the period subsequent to the administration was less than normal on this side. In experiment 13 the reverse occurred and at the end of ten minutes after the injection a small increase in secretion occurred. In experiment 2, an increase appeared more rapidly but as in this experiment one drop and only one occurred on the side where the chorda was cut it seems more probable that the increase in these cases were due to some uncontrolled reflex factor. Experiment 11 is perhaps even more conclusive, massive doses of iodides were given yet no secretion whatever occurred. As it might be suggested that the cutting of the chorda alone might lead to a constriction of blood vessels and in consequence a lack of secretion in experiment 30, both chorda and sympathetic were cut upon one side so that the gland was robbed of its nerve connections and yet no secretion on that side followed the administration of iodides. In experiment 7, a dog of 13 k. whose salivary center was depressed

neither an injection of 60 mm. of sodium iodide nor a subsequent injection of 300 mm. produced secretion. Pilocarpine however produced a good flow. It might be suggested that the presence of iodides would increase the amount of saliva excreted under reflex stimulation. This suggestion is shown not to be of any value by experiments 29 and 22. Experiment 27 shows that iodides taken into the stomach may serve to excite a flow of saliva reflexly and it is possible that in some subjects the nauseating taste of the iodide excreted into the mouth may excite an increased salivary flow.

Ammonium carbonate and ammonium chloride do not by their presence in the blood bring about a flow of saliva even when comparatively large quantities are given if the center is depressed or the chorda severed: such experiments as 30 and 22 serve to make this evident. In experiment 22, a slight increase is obtained when ammonium carbonate is given intravenously which is much exceeded by the effect obtained in experiment 30. It is evident that the action of these salts is not upon the gland but upon the center. Their reflex action is evidently more marked than is their action intravenously, (experiments 22 and 30). This conclusion seems somewhat at variance with the results of Salaskin (5) who found 2.56 mgm. ammonia in the saliva as compared with 1.12 in the blood. If his results are accurate this could only be explained by a selective excretion which does not seem to occur nor as our experiments have shown with sulphocyanates, nitrites or iodides, (experiment 30).

Ipecacuanha and emetine (experiments 23, 24 and 27) appear to have both a central and a reflex action though none on the gland peripherally or on the nerve endings in the gland. The reflex action is apparently the more important.

Antimony acts reflexly (experiments 28 and 30) and not upon the center. Both emetine and antimony had such marked heart actions when given intravenously that only small doses could be used and a slight central action might be readily overlooked.

Apomorphine has an action upon the center as may be seen in experiment 11; it has evidently no action upon the nerve endings

in the gland or upon the gland itself. Rossbach cited by Binz states that apomorphine causes bronchial secretion due to a direct stimulation in the glands. This is certainly not the case for the salivary glands.

The action of such drugs as pilocarpine, physostigmine and atrophine are not discussed as it is already well known.

CONCLUSIONS

1. Iodides are excreted by the salivary gland, but their presence in the blood stream does not initiate a flow of saliva if the salivary center is depressed or its nerve connections to the glands broken; nor does the excretion of iodides increase a previously existing flow or increase the effectiveness of a reflex stimulation. Salivation from iodides must be due to some reflex cause.

2. Other salts, sulphocyanates, carbonates, nitrites, when injected intravenously act similarly to iodides.

3. Emetine has a central and a reflex action.

4. Antimony in non-toxic doses acts only reflexly.

5. Ammonium salts have a central action but also a reflex action which is probably the more important.

6. Apomorphine acts directly upon the center. It has no peripheral action in the gland.

7. The salivary center is very labile, readily affected by sensory stimuli and readily depressed by narcotics.

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PROTOCOLS

Experiment 1. Dog, male 5.89 Kg. Urethane peritoneally and intravenously in all 9 2 gm. Chloroform during operation.

2.40, operation complete, left lingual cut above the chorda.

2.50-3.10, right 8.6 cc. in 20 min., left nothing. B. P. 90 mm. Temp. 38.2° C.

3.10-3.30, right 8 cc. in 20 min., left one drop.

3.37-25 mgm NaI as a 1 per cent solution given intravenously.

3.37-3.57, right 4.8 cc. in 20 min; left 2 drops: from 3.39-3.47, breathing was harder and the animal made slight movements. During the more forced breathing saliva flowed a little more rapidly for 3 min. At 3.55, 1.2 g. urethane given in two doses.

4.20, Pilocarp. Good flow. 3.57-4.17, right 2 cc., left 1 drop. B. P. 80 mm. Temp. 38.2° C. Iodide intravenously did not increase flow.

Experiment 2. Dog, female 9.52 Kg. A. C. E. Intracerebral Mg Cl₂.

11.00 a.m., operation complete. B. P. 115 mm.

11.00-11.20, right 1 cc., left chorda cut.

11.20-11.40, right 0.8 cc., left no flow.

11.40-12.00, right 0.4 cc. in 20 min., left no flow.

12.00-12.25, right 1.4 cc. in 25 min., left no flow.

12.25-12.50, 1.6 cc., left no flow.

12.55, 110 mgm., NaI in 10 per cent solution.

12.55-1.20, 2.1 cc. in 25 min., several drops in rapid succession within two minutes of injection, left one drop.

1.20-1.45, 1.7 cc. in 25 min., left no flow.

1.45-2.10, 1.6 cc. in 25 min., left no flow.

2.10-2.35, 0.1 cc. in 25 min., left no flow.

2.40, 110 mgm., NaI in 10 per cent solution.

2.40-3.05, 0.1 cc.

3.05-3.55, nothing; 3.55, 40 mgm. NaI. B. P. 110 mm.

3.55-4.20, nothing; 4.24, pilocarpine 1 mgm., good flow on both sides, secretion contained iodine. Voluntary respirations occurred after removal of pump. First injection of iodides seemed to increase salivary flow, that the increase was really due to a central action seems probable from the effect on the left side where one drop and one only appeared. Subsequent injections were without effect. Gland not damaged as pilocarpine caused good flow.

Experiment 5. Dog, female 4.89 Kg. Ether, magnesium chloride intracerebrally.

6.15, no flow of saliva during past 30 minutes. B. P. 80 mm. 11 cc. 1 per cent NaCl solution = 110 mgm. NaCl. at 37°C. one drop saliva fell in 4 minutes, one in the next 6 minutes, one in the following 15 minutes.

6.39, repeated the injection but at a temperature of 60°C. B. P. rose to 100 mm., drops in 1½ min., 2 min., 2 min., 2½ min., in 10 minutes a total of eight drops.

7.40, in last 60 min., 1.55 cc. One drop in last 5 minutes. B. P. 72 mm.

7.43, 10 cc. 1 per cent NaCl at 37°C. Drops in succeeding 5 min., 5 min., 5½ min., 6½ min.

8.05, 20 per cent mgm. NaI in 1 per cent solution at 37°C. Drops in succeeding 6½, 4; 4½, 5, 6; 10 cc. of normal saline did cause a slight flow in the first case but no increase in flow subsequently. The heat of the second injection of saline either acting directly or indirectly caused an increased flow. A small dose of iodide in warm solution had a like effect.

Experiment 6. Dog, female 13.0 Kg., A. C. E. and intracerebral MgCl₂.

10.45, drop of saliva in 37 sec. B. P. 140 mm. Hg.

10.45-11.05, 1.5 cc. in 20 min., drop in 30-35 seconds.

11.15, drop in 40-45 seconds.

11.05-11.25, 2.5 cc. in 20 min., drop in 35-40 sec. B. P., 135 mm. Hg.

11.30, 10 cc., 1 per cent NaCl intravenously.

11.33, drop in 33-35 seconds.

11.37, drop in 45-50 seconds.

11.25-11.45, 2.2 cc. in 20 min., drop in 45-47 seconds. B. P. 135 mm. Hg. A flow with pilocarpine was then set up and collected for another purpose.

2.10. The rate of flow has been 25-35 seconds per drop during past 25 minutes.

2.10-2.14, 80 cc. of 1 per cent NaCl at 40°C. drops during this time 30, 25, 20, 15, 15, 20.

2.18-2.20, drops in 28, 33, 37, 33 seconds. First injection of saline caused no increase in rate of flow, second and larger injection a slight increase.

Experiment 11. Dog, female 14.5 Kg. E. C. intracerebral MgCl₂ artificial respiration.

10.30-10.50, not a complete drop. B. P. 95-100.

10.50, 20 cc. Ringer's solution at 37°C., from this injection no flow resulted.

11.10, 250 mgm. NaI as a 10 per cent solution; from this injection no flow resulted.

11.30, 600 mgm. NaI as a 10 per cent solution; from this injection no flow resulted.

1.40, acid to mucous membrane of mouth, flow on both sides.

2.20-2.50, 0.4 cc. in 30 min., right chorda cut. B. P. 110 mm.

2.50, 5 mgm. apomorphine intravenously.

2.50-2.55, 2.2 cc. in 5 min. left; one drop right.

2.55-3.05, 5.8 cc. in 10 min. left; one drop right. Ringer's solution ineffective. Iodides also. Apomorphine acts centrally and not on endings in gland.

Experiment 12. Cat, 2.4. Intracerebral MgCl₂. Cut left sympathetic and chorda.

11.30-12.00, nothing, though central end of the lingual and of the IX and the nerves were stimulated and ether blast used. B. P. mm. 150.

12.00, 5 mgm. apomorphine intravenously. B. P. 130 mm.

12.15, 10 mgm. apomorphine intravenously, nothing or one partial drop on right side.

12.25, 5 mgm. apomorphine intravenously, nothing. B. P. 130 mm.

12.40, nothing left: 2 drops in right. B. P. 110 mm.

12.40, pilocarpine 2 mgm. intravenously.

12.45, in 5 min. 2.2 cc. left, and 2 cc. right. B. P. 100 mm. Apomorphine does not act if the center is depressed.

Experiment 19. Dog, male, 11.79 Kg. E. C. pithing, artificial respiration.

10.55-11.10, ether given in small amount by tracheal tube, left 4.8 cc., right 5 cc. in 15 min.

11.10-11.20, ether taken off, left 1 cc., right 0.8 cc. in 10 min.

11.23, drops falling in 65 to 71 seconds. A little (0.5 cc.) ether sprayed with a hypodermic needle into blast connection to trachea. Six drops in following 22 seconds, then slowing following drops in 55, 61, 68, 72 seconds, used for other purposes till 12.50 when the right chorda was cut. Ether blast produced several drops on left but none on the right.

12.50-1.05, in spite of stimulation, on right nothing, left 0.8 cc. in 15 min. B. P. 160.

1.05, 3 mgm. apomorphine intravenously.

1.05-1.07, right nothing, left 1.4 cc. in 2 min. B. P. 140.

1.12, movements resembling vomiting. Ether into trachea brings about a salivary flow. Apomorphine produces flow by acting on the center.

Experiment 20. Dog, male 13 K. 13 g. urethane and A. C. E. for operation.

3.00 p.m., no flow from either side. B. P. 130.

3.10, no flow from either side; ether test two blasts no flow.

3.11, 2 mgm. strychnine intravenously.

3.20, no flow from either side, ether test two blasts 0.5 cc.

3.30, no flow from either side, ether test two blasts 0.2 cc.

3.32, 2 mgm. strychnine.

3.34, ether test one blast, 0.2 cc.

3.45, ether test two blasts 0.2 cc. B. P. 130.

3.47, right chorda cut.

4.00, apomorphine 0.65 gm., flow began on left side in three minutes.

4.00-4.05, one drop right, 1 cc. left.

4.10, pilocarpine produced a good flow on both sides. Strychnine increases the irritability of the center. Apomorphine acts on the center directly and not on the glands.

Experiment 22. Dog, female, 4.89 Kg. E. C. urethane, right chorda cut Sherrington electrodes on right lingual.

11.03-11.08, right nothing, left 0.4 in 5 min. drops in 75 sec. B. P. 150. Secondary coil of inductorium at 13.

11.08, stimulated lingual for 3 min.: first drop in 35 sec. drops in 20, 22, 20, 25, 30, 20, 20, 20 (end of 3 min.) 20, 20, 15, 15, 18, 20, 25, 23, 28, 30, etc.

11.08-11.14, right one drop, left 1.4 in 6 min., 1.8 in 10 minutes.

11.14-11.18, right one drop; left 1.4 in 4 min.; 1.8 in 10 min.

11.18-11.28, right nothing, left 0.6 in 10 min.; 0.3 in 5 min.

11.29, 200 mgm. NaI in 10 per cent solution, slight movement accompanying injection.

11.28-11.33, left 0.4 in 5 min.

11.33, stimulated lingual for 3 min at 13; first drop in 5, second drops in 8, 8, 8, 8, 10, 16, 15, 16, 20, 25, 27, 35 end of 3 min., 55, 65.

11.33-11.38, right one drop; left 1 cc. in 5 min.

11.38-11.43, right nothing; left 0.4 cc. in 5 min.

11.43-11.48, nothing; left 0.6 in 5 min.; some stimulation during this period.

11.48-11.53, nothing; left 0.5 in 5 min.

11.53-12.03-12.08, nothing; left 0.5 in each 5 min.

12.10, 4 cc. 20 per cent ammon. carb. into stomach, drops in 20, 10, 5, 6, 6, 10, 12, 8, 9, 8, 9, 9, 11, 13, 17, 10, 13, 14, etc.

12.10-12.15, right one drop; left 2 cc. in 5 min.

12.15-12.20, right one drop; left 0.8 in 5 min.

12.20-12.25, right nothing; left 0.4 in 5 min. B. P. 120.

12.27, 2 cc. 20 per cent ammon. carb. intravenously.

12.25-12.30, right one drop, left 0.6 in 5 min.

12.30-12.35, right one half drop; left 0.4 in 5 min.

The first stimulation of the lingual produced a reflex secretion in 35 seconds, in the second case it appeared in five seconds. The secretion considerably outlasted the first stimulation and indeed was more rapid after its cessation. This was not true in the second case. The presence of iodide had evidently not increased the efficacy of the stimulation. Ammonium carbonate produces a secretion reflexly and by a direct action on the center, but no effect upon the gland directly.

Experiment 23. Dog, male 6.5 Kg., E. C. urethane, right chorda cut.

11.45-11.50, 0.4 cc. in 5 min. left; right about 0.01 cc. B. P. 135 mm.

11.50-11.55, 0.4 cc. in 5 min. left; right about 0.01 cc.

11.55, 3 cc. wine of ipecac. into stomach.

11.55-12.00, 1.0 cc. in 5 min.; right about 0.1 cc. B. P. 130 mm.

Ipecacuanha caused a flow reflexly. About 10 times as much on side with intact chorda as on that with sympathetic alone, previously the flow was roughly 40 times as great. Had there been an action on the nerve endings in the gland or on the gland itself the flow would have been more equal.

Experiment 24. Dog, female 5.89 Kg., E. C. urethane, right chorda cut.

10.40-10.55, left 0.2 cc in 15 min.; right 0.04 cc. B. P. 130 mm.

10.55-11.00, electrical and ether stimuli, left 0.4 cc. in 5 min.

11.00, 1 cc. fl. extract ipecac. intravenously.

11.00-11.10, left 1.2 cc. in 10 min; right 0.05 cc.

11.10-11.15, left 0.1 cc. in 5 min.; right 0. B. P. 90 mm.

11.15, 1 cc. fl. ext. ipecac. into stomach.

11.15-11.20, left 0.7 cc. in 5 min, right 0.05 in 10 min. B. P. 90 mm.

11.20-11.25, left 0.5 in 5 min.

Ipecac. intravenously and per os caused flow: when the fall in blood pressure is considered, its reflex action appears better than its central.

Experiment 27. Dog, male, 17.23 Kg. E. C. urethane, right chorda cut.

3.05-3.10, 1 cc. on left, nothing right. Drops in 31 and 33 seconds. B. P. 170 mm.
 3.10, 7 cc. 0.5 per cent HCl administered by stomach tube, rapid flow, left, drops in 8, 10, 6, 4, 3 seconds.

3.10-3.15, 2.2 cc. in 5 min.

3.15-3.20, 3.6 in 5 min.

3.20-3.25, 3.2 in 5 min.

3.25-3.30, 2.1 in 5 min.; from 3.10-3.20 one drop formed and fell on right side.

3.32, drops were falling at rate of one in 15, 14, 13, 14, 15, right crural nerve stimulated for 30 seconds; drops now 4 in 15 sec., 2 in 20, 2 in 21, then slowing to previous rate; one drop on right side. one-half was formed before stimulation began.

3.30-3.35, 1.6 in 5 min. B. P. 160 mm.

3.35-3.40, 1.2 in 5 min.

3.40-3.45, 0.6 in 1 min.

3.45, 2 cc. 10 per cent sodium iodide by stomach tube.

3.45-3.50, 1.2 cc. in 5 min; 3.30-3.45, one-half drop formed on right side.

3.50-3.55, 1 cc. in 5 min.

3.55-4.00, 1 cc. in 5 min.

4.00-4.05, 0.6 cc. in 5 min. B. P. 1.55 mm.

4.05, 6 mgm. emetine intravenously.

4.05-4.10, 1.0 cc. in 5 min; drop fell on right side.

4.10-4.15, 1.0 cc., in 5 min; drop fell on right side.

4.15-4.20, 0.6 cc. in 5 min.

4.20-4.25, 0.6 cc.

4.25, 6 mgm. emetine intravenously.

4.25-4.30, 0.8 cc. in 5 min.; drop fell in right side.

4.30, 2 cc. fluid extract of ipecac. into stomach.

4.35-4.40, 0.8 in 5 min.

4.40-4.45, 0.5 cc. in 5 min.

4.45, 20 mgm. morphine intravenously.

4.45-5.05, nothing, stimulation of crural and of lingual centrally ineffective ether blast also.

5.05, pilocarpine, good flow.

5.20, artificial respiration stopped. Asphyxial respiratory movements. Emetine acts when given intravenously. Morphine prevents reflex flow. Stimulation of crural causes reflex flow. Sodium iodide *per os* caused flow.

Experiment 28. Dog, female 6.35 Kg. E. C. urethane, right superior sympathetic ganglion destroyed; left chorda cut.

10.23, drops falling on right side at rate of one in 15 seconds.

10.25, ether blast first drop fell in $2\frac{1}{2}$ sec. time of successive drops, 3, 4, 6 (7 drops in 30 sec., 12 drops in first minute) slowing to drop in 20; on left side $\frac{1}{2}$ drop. B. P. 170.

10.30, stimulated lingual for 30 seconds with secondary coil at 10, drops in 3, 7, 15, 20, 20, 24, 26, 25, 29 seconds.

10.35, stimulated crural for 30 seconds with secondary coil at 10, drops, 5, 15, 30, 30, 31, 32. During these two periods about $\frac{1}{2}$ drop formed on left side.

10.50, drops falling on right side about one in 15 seconds. 40 cc. Ringer's solution at 37°C. were given intravenously in 5 minutes; after 10 cc. had been given average rate per drop 15 sec. After 20 cc. 13, sec. after 30 cc. 13, after 40 cc. 14 sec.

11.00-11.05, right side 0.6 cc. in 5 min., left nothing.

11.05, 2 cc. 5 per cent ammon. sulphocyanate.

11.05-11.10, right 0.6 cc. in 5 min., left nothing.

11.10-11.15, right 0.6 cc. in 5 min., left nothing. B. P. 170-190.

11.18, 2 cc. wine of antimony into stomach.

11.15-11.20, right 1.0 cc in 5 min., left $\frac{1}{2}$ drop.

11.20-11.25, right 1.2 cc. in 5 min., left $\frac{1}{2}$ drop.

11.25-11.30, right 1.2 cc. in 5 min., left $\frac{1}{2}$ drop. B. P. 170.

11.30, 60 mgm. morphine intravenously, flow stopped completely.

11.43, pilocarpine caused good flow on both sides.

Lingual stimulation more efficient than crural. Lingual stimulation brings about prompt response (3 seconds latent period). Ether to tongue equally prompt. Reflex stimulation brings about 20-30 times more secretion when the chorda alone is intact than when the sympathetic alone is. Morphine cuts off central secretion.

Experiment 29. Dog, female 11.79 Kg. A. C. E. Intracerebral $MgCl_2$. Operation complete at 11.00 natural respiration through a tracheal cannula.

11.00-11.10, 0.6 cc.

11.10, ether blast.

11.10-11.20, 1.0 cc.

11.20-11.30, 0.6 cc.

11.20, 200 mgm. NaI.

11.30-11.40, 0.6 cc.

11.40-11.50, 0.8 cc.

11.50-12.00, 1 cc.

12.00, ether blast.

12.00-12.10, 1.3 cc.

12.10-12.20, 1 cc.

Increase due to ether stimulation not quite so great after as before iodides, in spite of the fact that the flow which must have been due to uncontrolled reflex stimulation was greater. The center was probably more active.

Experiment 30. Dog, male 10 Kg. Urethane 1 g. per kilo ether.

10.00-10.25, no secretion. B. P. 150-140 mm.

10.25, 2 cc., 20 per cent ammonium carbonate, slowly intravenously in succeeding two minutes 2 drops appeared on each side.

10.25-10.40, 2 drops.

10.40, 4 cc., same solution into stomach.

10.45, movements of abdomen resembling vomiting but not leading to vomiting for three minutes; 2 cc. in 3 min.

10.40-10.50, 5 cc. in 10 min. B. P. 150 mm.

10.50-11.00, 3.4 in 10 min.

11.03-11.10, several tests of the center were made with the ether method and it was shown to be active. This much increased the amount of saliva for this period.

11.00-11.10, 3.6 cc. in 10 min.

11.12, 4 cc. wine of antimony into stomach, no vomiting movements.

11.10-11.20, 4 cc. in 10 min. B. P. 120 mm.

Ammonium carbonate had little effect intravenously, marked effect *per os*. Reflex flow increased by antimony *per os*.

Experiment 31. Dog, male, 20 Kg, E. C. urethane. Right sympathetic cut. B. P. 95-100 mm.

10.45, flow on both sides approximately equal.

11.00, right chorda cut.

11.05, right nothing; left 0.4 cc. in 5 min.

11.10, right nothing; left 0.4 cc.

11.11, 100 mgm. sulphocyanate of sodium intravenously.

11.15, right nothing; left 0.2 cc.

11.20, right nothing; left 0.3 cc.

11.26, 200 mgm. sodium iodide intravenously.

11.25, right nothing; left 0.3 cc.

11.30, right nothing; left 0.3 cc.

11.31, 100 mgm. sodium nitrite intravenously, followed by barium chloride to restore the blood-pressure.

11.35, right nothing; left 0.4 cc. B. P. 110 mm.

11.40 right nothing; left 0.3 cc.

11.40 to 12.03, used for observations on the cilia of the trachea.

12.03, right nothing; left 0.6 in last 5 min.

12.08, right nothing; left 0.6.

12.13, right nothing; left 0.6.

12.14, 2 cc. 20 per cent ammonium chloride *per os*.

12.18, right nothing; left 1.1 cc.

12.23, right nothing; left 0.6 cc.

12.24, 200 mgm. ammonium carbonate intravenously.

12.28, right nothing; left 1.4 cc.

12.33, right nothing; left 1.4 cc.

12.38, right nothing; left 0.8 cc.

12.43, right nothing; left 0.6 cc.

12.44, 350 mgm. sulphocyanate intravenously.

12.48, right nothing; left 0.6 cc.

12.53, right nothing; left 0.6 cc. B. P. 90.

1.03, pilocarpine 2 mgm. caused an abundant flow 4 cc. from each side in 5 minutes.

Sulphocyanides, iodides, nitrites, when present in the blood do not stimulate salivary flow. Ammonium carbonate acts on the center but not on the gland-cells. Ammonium chloride has a reflex action.



THYREOTROPIC IODINE COMPOUNDS

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The thyroid gland has, as is well known, a special affinity for iodine. Not only does it take up and retain iodine from the minute amounts present in drinking water and ordinary foods, but it takes up and retains some of that administered in the form of potassium iodide and iodoform; its physiological activity may be greatly and rapidly increased in this manner.¹ The question whether certain iodine compounds have a special affinity for the thyroid, whether, in other words, there are thyreotropic iodine compounds, as Loeb,² for example, showed there are lipotropic iodine compounds, does not seem to have been investigated; Kocher stated several years ago that such a compound is a great desideratum in the treatment of goitre.

We believe we have found in bladderwrack an iodine compound which may properly be called thyreotropic, that is, a compound which, as we interpret our results, increases the activity of the thyroid in doses far smaller than do any other iodine compounds with which we have experimented.

We have approached the subject from the physiological rather than from the chemical or analytical standpoint; that is, we have endeavored to test the effects of various compounds upon the activity of the thyroid instead of determining how much iodine is actually absorbed by the gland. The latter method is open to

¹ Hunt and Seidell, Studies on Thyroid. 1. The relation of iodine to the physiological activity of thyroid preparations, Bull. 47, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service, Washington, D. C., 1909.

² O. Loeb, Archiv. Exper. Path. u. Pharm., 1906, 56, p. 320.

the criticism that the mere presence of iodine in the thyroid is no evidence of its physiological importance. In fact, some of the more recent writers who have approached the subject from the analytical point of view have expressed doubts whether any physiological significance is to be attached to the presence of iodine in the thyroid.³

We recognize that our method also is open to criticism, and that the results should be confirmed by analytical and certain physiological experiments which we are unable at present to perform; but, although the exact interpretation of our results may be open to question, we believe that we have discovered certain facts of some interest in connection with the pharmacology of iodine.

The considerations which led to our experiments were summarized by one of us in an earlier paper as follows:⁴

a. *The Effects of Certain Iodine Compounds are in Part the Same as Those of Thyroid. Experiments with Acetonitrile.* Many experiments showed that potassium iodide and some other iodine compounds (bladderwrack, iodoform, iodole, etc.) when fed to mice increase their resistance to acetonitrile; thyroid has a similar but far greater effect. This fact would not have much significance were it not that rats and guinea-pigs (if the thyroid gland is intact) react towards these iodine compounds in exactly the opposite way, but still just as they do towards thyroid; in other words, the resistance of rats and guinea-pigs towards acetonitrile is lowered, or at least never increased, by feeding thyroid and certain iodine compounds.

In some experiments the administration of iodine compounds had either a very slight or no effect. It is probable that this absence of effect was due to the condition of the thyroid. The point which I desire to emphasize here, however, is that whenever potassium iodide or other iodine compound does have an effect on the resistance of animals to acetonitrile (and this is usually the case) it invariably affects mice and guinea-pigs in exactly opposite directions.

b. *Experiments with Morphine.* The feeding of thyroid lowers the resistance of mice, rats and guinea-pigs to morphine; the feeding of potassium iodide and bladderwrack has a similar though less marked

³ The literature on this subject has recently been reviewed by us in the Bulletin quoted above.

⁴ Hunt, Journal Amer. Med. Assoc., 1907, 49, p. 1323.

effect; here again the iodine compounds affected the resistance of animals to this poison the same way that thyroid did.

It was further shown that the effect of several iodine compounds upon the resistance of guinea-pigs to acetonitrile was either greatly diminished or entirely abolished if the thyroids had been removed; the following conclusions were drawn:

The iodine compounds convert the available iodine-poor thyreoglobulin into an iodine-rich compound, with the result that the animal is in a condition of hyperthyroidism; it was shown above that when such a condition of hyperthyroidism is produced by the feeding of thyroid the animals show the same increased susceptibility to acetonitrile.

It is impossible to increase the susceptibility by the administration of iodine compounds beyond a certain point, and this is less than that which may be produced by thyroid feeding; this difference probably depends on the fact that the thyroid has at any time but a limited supply of iodine-poor thyreoglobulin; when this is iodized to its maximum there can be no further increase in susceptibility. Of course, it is possible that another factor is involved: that the effect of the iodine is simply to cause an increased secretion of the active principle already present, but on the whole the former suggestion seems more probable.

The plan of the following experiments was to determine the smallest amounts of various iodine compounds which when fed to animals, caused distinct changes in their resistance to acetonitrile, and also to determine the maximum effect which could be obtained. If the hypothesis that certain compounds are thyreotropic is correct, we should find that these cause a change in the resistance in much smaller doses in terms of their iodine content than the other compounds. If, further, iodine compounds affect the resistance of animals to acetonitrile only through the thyroid gland, then we should not expect to find that the maximum effect of one compound would differ markedly from that of others. As will be shown, the experiments are in accord with the hypothesis in both respects.

It was thought that, in addition to the light such experiments would throw on the existence of thyreotropic iodine compounds, they would be of interest in other connections. Thus so far no other iodine compound has been found which has the effects

upon metabolism, cretinism or myxœdema that thyroid has; as will be shown, we also, making use of this new and extremely delicate test for thyroid, have found no other iodine compound having the effects of thyroid. Further, we have already shown that the physiological test for thyroid is far more delicate than any chemical test, and have suggested that use might be made of it in testing the blood in cases of exophthalmic goitre, for detecting thyroid in anti-fat nostrums, etc., but to do this it was desirable to determine more exactly the effects of other iodine compounds.

EXPERIMENTAL.

a. Experiments on Mice. Nearly all of the mice used in these experiments were bred in the laboratory and had been kept under as uniform conditions as regards diet⁵ temperature, etc., as possible. The iodine compounds were fed in the form of cakes according to Ehrlich's method; the feeding was continued for from 9 to 12 days; it is believed that the maximum effect of the iodine compounds is obtained by feeding for this period although the effects of the previous diet may not be entirely overcome; the latter may, however, be overlooked, for the previous diet had been the same for all of the mice of a given series.⁶

The cakes weighed about 4 grams each and the figures given in the protocols indicate the weight of the drug in each cake. These weights, multiplied by the percentage of iodine in the several compounds, give the milligrams of iodine fed per cake.

⁵ This precaution is essential, for diet has a profound effect not only upon the resistance of mice to acetonitrile but also upon the degree to which the resistance is modified by iodine compounds. In fact, far more marked effects may be obtained by changes in diet than by the administration of iodine compounds or of any other drugs (except thyroid); further, the character of the diet has more influence upon the result of the administration of iodine compounds than the character of the latter. In other words more marked results are obtained by modification of the "iodine receptors" of the thyroid by diet than by the administration of thyreotropic iodine compounds. This subject is more fully discussed in Bulletin 69 of the Hygienic Laboratory now in press.

⁶ The wide variations in the fatal doses for the control in the protocols of the following experiments are probably due in part to the earlier diet and in part to the season and to the age of the mice.

The acetonitrile was dissolved in water and injected subcutaneously. Many experiments were performed upon each group of mice, but for the sake of brevity only the largest non-fatal and the smallest fatal doses are given in the protocols.

SERIES I (SEPTEMBER, 1908) (XI. 14)

DRUG	PER CENT IODINE IN DRUG	MG. IODINE PER CAKE	FATAL DOSE ACETONITRILE IN MG. PER GM. MOUSE	
			RECOVERED	DIED
a.—Controls.....			0.2	0 25
b.—Extract of bladderwrack (<i>Fucus vesiculosus</i>) 5 mgm.....	0.11	0.0055	0.23
c.—Bladderwrack, 1 mgm.....	0.11	0.0011	0.2	0.28
d.—Hemol-iodo, ⁷ 1 mgm ⁷	16.00	0.16	0.17	0.21
e.—Iodalia, ⁸ 1 mgm.....	1.05	0.0105	0.34	0.4
f.—Iodo eosin, 1 mgm.....	60.75	0.61	0.2	0.23
g.—Iodoform, 1 mgm.....	96.7	0.97	0.26	0.27
h.—Sajodin, 1 mgm.....	24.5	0.245	0.2	0.26
i.—Thyroid, ^a 1 mgm.....	0.065	0.00065	0.5	0.58
j.—Thyroid, 5 mgm.....	0.065	0.0033	1.9	2.0
k.—Thyroid, 1 mgm.....	0.38	0.0038	1.8	2.0
l.—Thyroid, 5 mgm.....	0.38	0.0190	4.2

a Desiccated thyroid used in all cases.

Summary of Series I. The only iodine compounds aside from the thyroid which protected against acetonitrile were (e) iodalia and (g) iodoform. Iodalia containing 0.0105 mgm. iodine protected against about $1\frac{1}{2}$ times the dose fatal to the controls.

Iodoform containing 0.97 mgm. iodine protected against perhaps $1\frac{1}{2}$ times the fatal dose; thyroid containing but $\frac{1}{1500}$ as much iodine protected against twice as much; hence we may estimate the iodine of the latter as being about 3,000 times as active as that of the former. Thyroid containing 0.0190 mgm. iodine protected against at least 21 times the fatal dose.

⁷ This is stated to be Hemol (see New and Non-Official Remedies, 1910, p. 101) with 16 per cent iodine organically combined.

⁸ This is stated to be "iodotannic saccharate" and to contain in 5 gms. 0.06 gm. iodine combined with tannin. We found approximately the percentage of iodine claimed.

SERIES II (FEBRUARY, 1907) (VII. 192)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE.	
			RECOVERED	DIED
a.—Cakes.....	0.37	0.4
b.—Bladderwrack, 0.01 gm.....	0.11	0.011	0.45	0.7

Summary. Bladderwrack containing 0.011 mgm. iodine had a slight effect in protecting mice against acetonitrile.

SERIES III (JULY, 1907) (VII. 82)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE.	
			RECOVERED	DIED
a.—Controls.....	0.31	0.32
b.—Bladderwrack, 20 mgms.....	0.084	0.0168	0.47	0.49
c.—Iodalbin; ⁹ 0.1 gm.....	21.0	21.0	0.47	0.51
d.—Iodoform, 0.02 gm.....	96.7	19.34	0.45	0.47
e.—Iodole, 0.01 gm.....	88.96	8.9	0.46	0.51
f.—Iodonucleoid, ¹⁰ 0.2 gm.....	23.00	46.0	0.46	0.51
g.—Potassium iodide, 0.001 gm.....	76.4	0.76	0.38	0.41
h.—Sajodin, 0.05 gm.....	24.5	12.25	0.32	0.35

Summary. Bladderwrack, iodalbin, iodoform, iodole, and iodonucleoid all protected mice against about $1\frac{1}{2}$ times the dose of acetonitrile fatal to the controls. It is probable that this was the maximum protection possible with these mice; hence it is difficult to draw conclusions as to the relative efficiency of the different forms of iodine. 0.76 mgm. iodine in potassium iodide was distinctly less efficient than 0.0168 mgm. iodine in bladderwrack;

⁹ Iodalbin is a compound of iodine and blood albumin, containing approximately 21.5 per cent iodine (New and Non-Official Remedies, 1910, p. 109). The sample used in these experiments contained 21 per cent iodine.

¹⁰ This is a proprietary preparation claimed (at least at the time these experiments were made) to be a compound of iodine and nuclein. It is apparently a compound of iodine with casein, not with a true nuclein. It was formerly made with 10 per cent iodine. Later it was stated to contain 23 per cent iodine in organic combination and this is the percentage we have found in the samples we used; a small part of this is evidently not in organic combination as will be shown later.

we may estimate the latter to be more than 45 times as active as the former. 12.25 mgm. iodine in sajodin had almost no effect; we can estimate the efficiency of the iodine in bladderwrack as being about 700 times as great as that of sajodin.

SERIES IV (SEPTEMBER, 1908) (XI. 66)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MG. PER GM. MOUSE.	
			RECOVERED	DIED
a.—Controls.....	0.2	0.22
b.—Bladderwrack, 16.6 mgm.....	0.087	0.014	0.3	0.35
c.— { Potassium iodide, 0.0093 mgm...	76.5	0.014	0.27	0.35
{ Bladderwrack, 8.3 mgm.....	0.087			
d.—Iodalbin, 0.5 mgm.....	21.0	0.105	0.2
e.— { Iodalbin, 0.0335 mgm.....	21.0	0.014	0.22
{ Bladderwrack, 8.3 mgm.....	0.087			
f.—Thyroid, 1 mgm.....	0.121	0.0012	1.4	1.7
g.—Thyroid, 1 mgm.....	0.2	0.002	1.6	1.7

Summary of Series IV. b. Bladderwrack containing 0.014 mgm. iodine protected against about $1\frac{1}{2}$ times the fatal dose of acetoni-trile. d. Iodalbin containing 8 times as much iodine had no effect. f. Thyroid containing 0.0012 iodine protected against about 7 times the fatal dose. Since the amount of bladderwrack fed contained nearly 12 times as much iodine as the thyroid we may estimate the latter as being about 60 times as active as the former.

When the iodine was administered partly in bladderwrack and partly in potassium iodide there was a low degree of protection; a similar combination of iodalbin and bladderwrack had no effect.

SERIES V (OCTOBER, 1908) (XI. 74)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE.	
			RECOVERED	DIED
a.—Controls.....	0.36	0.37
b.—Bladderwrack, 0.1 gm.....	0.03	0.03	0.48	0.51
c.—Bladderwrack, 0.3 gm.....	0.03	0.09	0.46	0.50
d.—{ Bladderwrack, 0.1 gm.....	0.03	2.97	0.5	0.54
{ Sajodin, 0.012 gm.....	24.5			
e.—{ Bladderwrack, 0.3 gm.....	0.03	3.03	0.5	0.51
{ Sajodin, 0.012 gm.....	24.5			
f.—Iodalia, 0.1 mgm.....	1.05	0.001	0.41	0.42
g.—Iodoform, 0.1 mgm.....	96.7	0.097	0.36	0.38
h.—Sajodin, 12 mgm.....	24.5	2.94	0.56	0.6
i.—Thyroid, 1 mgm.....	0.18	0.0018	1.7

Summary. Bladderwrack, bladderwrack and sajodin, and sajodin, increased the resistance by one-half or more. Iodalia had a slight effect. Bladderwrack with 0.03 mgm. iodine was as effective as that with 0.09 mgm.; the effect was but slightly increased when a relatively large amount of sajodin was added. Thyroid containing 0.0018 mgm. iodine was at least 3 times as effective as bladderwrack containing 0.03 mgm. iodine; hence the thyroid was at least 50 times as active as bladderwrack. The activity of the thyroid may be similarly estimated as at least 5000 times as great as that of sajodin and the activity of bladderwrack as about 90 times as great as that of sajodin (all expressed in terms of iodine).

SERIES VI (MAY, 1907) (VII. 128)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE.	
			RECOVERED	DIED
a.—Controls.....	0.22	0.23
b.—Bladderwrack (purified) 0.012 gm.	0.33	0.0396	0.29	0.31
c.—Bladderwrack, 0.05 gm.....	0.082	0.0411	0.26	0.28

Summary. The bladderwrack protected against nearly $1\frac{1}{2}$ times the fatal dose.

SERIES VII (MAY, 1908) (x. 164)

DRUG	PER CENT IODINE IN DRUG	MG. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MG. PER GM. MOUSE.	
			RECOVERED	DIED
a.—Controls.....	0.56	0.58
b.—Bladderwrack, 0.05 gm.....	0.082	0.041	0.6	0.65
c.—Iodothyrim ¹¹ A, 0.2 mgm.....	0.38	0.00076	1.7	2.1
d.—Thyroid, 0.5 mgm.....	0.11	0.00055	2.9	3.3
e.—Thyroid, 1.0 mgm.....	0.12	0.0012	4.6	4.9

Summary. Extract of bladderwrack containing 0.041 mgm. iodine protected against perhaps $1\frac{1}{10}$ times the fatal dose of acetonitrile. Thyroid containing 0.00055 mgm. iodine protected against $5\frac{1}{2}$ times the fatal dose. The iodine in the thyroid was about 400 times as active as that in the bladderwrack. The iodine in our crude iodothyrim was not quite as active as that of the thyroid but it was far more active than that of the bladderwrack.

SERIES VIII (MARCH, 1908) (x. 114)

DRUG	PER CENT IODINE IN DRUG	MG. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MG. PER GM. MOUSE.	
			RECOVERED	DIED
a.—Controls.....	0.43	0.46
b.—Bladderwrack, 0.05 gm.....	0.086	0.043	0.58
c.—Iodothyrim (Bayer) (31) ¹² 5.0 mgm.	0.014	0.0007	2.0	2.2
d.—Iodothyrim (Bayer) (32) 5.0 mgm..	0.015	0.00075	0.9
e.—Iodothyrim (Bayer) (764a) 5.0 mgm.	0.019	0.00095	1.8	2.0
f.—Thyroid, 1.0 mgm.....	0.19	0.0019	3.8	4.3

Summary. Extract of bladderwrack containing 0.043 mgm. iodine protected against at least $1\frac{1}{3}$ times the fatal dose. Thyroid containing 0.0019 mgm. iodine protected against 9 times the fatal dose. We may estimate the iodine of the thyroid as being about 150 times as active as that of the bladderwrack. Two of

¹¹ Crude iodothyrim prepared in the laboratory.

¹² This and the two following were commercial preparations of Bayer and Co. This iodothyrim is stated to be a milk sugar trituration of the active principle

the samples of iodothyrim were as active as the thyroid; the other one was much less active.¹³

SERIES IX (DECEMBER, 1907) (X. 80)

DRUG.	PER CENT IODINE IN DRUG.	MGM. IODINE PER CAKE.	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE.	
			RECOVERED.	DIED.
a.—Controls.....	0.17	0.19
b.—Bladderwrack, 0.05 gm.....	0.086	0.043	0.22
c.—Iodalbum, 0.01 gm.....	21.0	2.1	0.25	0.27
d.—Iodalia, 0.1 gm.....	1.05	1.05	0.33	0.34
e.—Thyroid, 2.0 mgm.....	0.17	0.0034	2.3	2.4
f.—Thyroid, 4.0 mgm.....	0.087	0.00348	2.3	2.5

Summary. It is doubtful whether the extract of bladderwrack containing 0.043 mgm. iodine had any effect. Iodalbum containing 2.1 mgm. iodine protected against less than $1\frac{1}{2}$ times the fatal dose. Iodalia containing 1.05 mgm. iodine protected against $1\frac{8}{10}$ times the fatal dose; Thyroid with 0.0034 mgm. iodine protected against 13 times the fatal dose. The iodine of iodalia seemed to be 2 or 3 times as effective as that of iodalbum; that of the thyroid seemed to be over 2,000 times as efficient as that of iodalia and 4 or 5 thousand times as effective as that of iodalbum.

of the thyroid gland 1 gm. of the substance corresponding to the average amount of iodine in 1 gm. of fresh sheep's thyroid (0.0003 gm.); this would be 0.03 per cent iodine; we found but 0.014 to 0.019 per cent in the three samples we examined. Preparation 32 which had a very low degree of physiological activity gave tests for loosely combined iodine; the iodine of the thyroid is, as well known, in a remarkably stable form of combination.

¹³ Pick and Pineles (Zeitsch. f. exper. Path. u. Ther., 1909, 7, p. 518) have recently tested the effect of iodothyrim upon myxœdematos kids; they state that their experiments afford no support to the view that iodothyrim is the only active or one of the active principles of the thyroid. They do not seem to have examined the iodothyrim they used to determine if the iodine was in firm combination; without such examination we do not believe their conclusions are justified. (cf for example, Hunt and Seidell, Commercial Thyroid Preparations, etc., Jour. Amer. Med. Assoc. 1908, 51, p. 1385). We are not convinced that "iodothyrim" is a definite compound and we do not believe that it represents the secretion of the thyroid, *in vivo*, but we do believe that it is possible to prepare from thyroid "iodothyrim" which does represent the full activity of the gland when given per os; some preparations however, seem to contain less of it than others probably as a result of the variable conditions under which they have been prepared.

SERIES X (AUGUST, 1908) (XI. 54)

DRUG.	PER CENT. IODINE IN DRUG.	MGM. IODINE PER CAKE.	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE.	
			RECOVERED.	DIED.
a.—Controls.....	0.23	0.24
b.—Bladderwrack, 10.0 mgm.....	0.084	0.0084	0.34	0.36
c.— { Bladderwrack, 10.0 mgm.....	0.084	1.06	0.50	0.51
{ Iodalbin, 5.0 mgm.....	21.			
d.—Iodalbin, 5.0 mgm.....	21.0	1.05	0.24	0.26
e.— { Bladderwrack, 10.0 mgm.....	0.084	3.8284	0.46	0.47
{ Potassium iodide, 5.0 mgm.....	76.5			
f.—Potassium iodide, 5.0 mgm.....	76.5	3.82	0.28	0.30
g.—Thyroid, 1.0 mgm.....	0.12	0.0012	0.85	1.00
h.—Thyroid, 1.0 mgm.....	0.2	0.002	1.7	1.8

Summary. Extract of bladderwrack with 0.0084 mgm. iodine protected against $1\frac{1}{2}$ times the fatal dose; iodalbin with 1.05 mgm. iodine (or 125 times as much) had no distinct effect. Hence we may estimate that the iodine of bladderwrack is about 180 times as active as that of iodalbin. Potassium iodide containing 3.82 mgm. iodine protected against $1\frac{1}{2}$ times the fatal dose; this was less than that caused by bladderwrack containing 0.0084 mgm. (or 450 times less) iodine. Hence we may estimate the iodine in bladderwrack as being about 500 times as active as that in potassium iodide. Thyroid containing 0.002 mgm. iodine protected against about $7\frac{2}{3}$ times the fatal dose; or it was about 5 times as active as bladderwrack containing more than 4 times as much iodine. Hence the iodine of thyroid in this series may be estimated as being about 20 times more active than that of bladderwrack and 3600 times as active as that of iodalbin and 10,000 times as active as that of potassium iodide.

It is interesting to note that although iodalbin and potassium iodide alone had, in the doses fed, scarcely any effect they very distinctly increased the activity of bladderwrack when added to the latter.

SERIES XI (JULY, 1907) (VII. 34)

DRUG.	PER CENT. IODINE IN DRUG.	MGM. IODINE PER CAKE.	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE.	
			RECOVERED.	DIED.
a.—Controls.....	0.38	0.39
b.—Bladderwrack, Ext. (purified). 12 mgm.....	1.41	0.17	0.42	0.43
c.—Potassium iodide, 10 mgm.....	76.4	7.6	0.39	0.40
d.—Thyroid, 5 mgm.....	0.136	0.0068	2.5

Summary. Bladderwrack containing 0.17 mgm. iodine had a slight protective action; potassium iodide with 7.6 mgm. iodine apparently had a very slight effect. We may estimate the iodine of the former as being about 50 times as effective as that of the latter. Thyroid with 0.0068 mgm. iodine protected against at least 6 times the fatal dose; the iodine of the thyroid may be estimated as being at least 150 times as active as that of the bladderwrack and at least 7500 times as active as that of potassium iodide.

SERIES XII¹⁴ (JANUARY, 1907) (VII. 146)

DRUG.	PER CENT. IODINE IN DRUG.	MGM. IODINE PER CAKE.	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE.	
			RECOVERED.	DIED.
a.—Controls	0.66	0.70
b.—Bladderwrack, 0.2 gm.....	0.11	0.22	1.8
c.—Iodoeosin, 0.02 gm.....	60.75	12.1	1.2	1.8
d.—Iodoform, 0.02 gm.....	96.7	19.34	0.8	1.3
e.—Iodole, 0.01 gm.....	88.96	8.9	1.5	2.0

Summary. Extract of bladderwrack containing 0.22 mgm. iodine protected against more than twice the fatal dose of acetonitrile. Iodoeosin containing 12.1 mgm. iodine protected mice against about twice the fatal dose. Iodoform with 19.34 mgm. iodine protected mice against perhaps $1\frac{1}{2}$ times the fatal dose of

¹⁴ These were old mice.

acetonitrile. Iodole with 8.9 mgm. iodine protected against more than twice the fatal dose. Thus the iodine in the bladderwrack seemed to be from 40 to 90 times as effective as that in the other forms of combination.

SERIES XIII (November, 1908) (XI 18)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGm. PER GM. MOUSE	
			RECOVERED	DIED
a. —Cakes.....	0.2	0.25
b. —Bladderwrack, 0.3 gm.....	0.084	0.252	0.29	0.31
{ Bladderwrack, 0.3 gm.	0.084
c. —{ Potassium iodide, 2 mgm.	76.5	1.782	0.42	0.45
d. —{ Bladderwrack, 0.3 gm.	0.084
{ Potassium iodide, 4 mgm.	76.4	0.65
e. —{ Bladderwrack, 0.3 gm.	0.084
{ Potassium iodide, 2 mgm.....	76.5	3.252	0.65	0.8
{ Sajodin, 6 mgm.	24.5
f. —{ Bladderwrack, 0.3 gm.	0.084
{ Sajodin, 6 mgm.	24.5	0.75
g. —{ Bladderwrack, 0.3 gm.	0.084
{ Sajodin, 12 mgm.	24.5	0.8
h. —Potassium iodide, 4 mgm.....	76.5	3.06	0.3	0.33
i. —{ Potassium iodide, 2 mgm.	76.5
{ Sajodin, 6 mgm.	24.5	3.0	0.35	0.38
j. —Sajodin, 12 mgm.....	24.5	2.94	0.25	0.3
k. —Thyroid, 1 mgm.....	0.2	0.002	2.3	2.7

Summary. Extract of bladderwrack with 0.252 mgm. iodine, potassium iodide with 3.06 mgm., and sajodin with 2.94 mgm.

protected mice against from about $1\frac{1}{2}$ to $1\frac{2}{3}$ times the fatal dose of acetonitrile. Thyroid containing 0.002 mgm. iodine protected against about 11 times the fatal dose; the iodine in the thyroid was evidently thousands of times more active than that in the other compounds. The combination of bladderwrack with potassium iodide and still more that with sajodin caused a relatively high degree of resistance; the effect was less marked with potassium iodide and sajodin. The maximum degree of protection obtained by these combinations probably did not exceed 4 times the fatal dose.

SERIES XIV (MAY, 1908) (x. 160)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.54	0.55
b. —Potassium iodide, 0.26 mgm.....	76.5	0.199	0.55	0.58
c. —Thyroid, 0.5 mgm.....	0.106	0.00053	1.9	2.1
d. —Thyroid, 0.5 mgm.....	0.3	0.0015	3.7	4.2

Summary. b. Potassium iodide containing 0.199 mgm. iodine had no appreciable effect upon the resistance of mice to acetonitrile. c. Thyroid containing 0.00053 mgm. iodine (or nearly 375 times less) protected against $3\frac{1}{2}$ times the fatal dose; the iodine in the thyroid was at least 1300 times as active as that in potassium iodide. d. Thyroid containing 0.0015 mgm. iodine protected against about $7\frac{1}{2}$ times the fatal dose; the dose was probably larger than the one necessary to produce the maximum protection.

SERIES XV (APRIL, 1908)(x. 156)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			DIED	RECOVERED
a. —Controls.....	0.72	0.75
b. —Potassium iodide, 2.6 mgm.....	76.5	1.99	0.9	1.2
c. —Thyroid, 1 mgm.	0.106	0.00106	2.0	2.3
d. —Thyroid, 1 mgm.....	0.3	0.003	3.0	3.2

Summary. b. Potassium iodide containing 1.99 mgm. iodine had a very slight effect; perhaps it protected against about $1\frac{1}{2}$ times the fatal dose of acetonitrile. c. Thyroid with 0.00106 mgm. iodine (or 1900 times less) protected against nearly 3 times the fatal dose. d. Thyroid with 0.003 mgm. iodine protected against about $4\frac{1}{2}$ times the fatal dose. Thus the iodine of thyroid was from 2000 to 4000 times more active than that of potassium iodide.

SERIES XVI (JULY, 1907)(VII. 42)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MG. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.37	0.4
b. —Potassium iodide, 5 mgm.....	76.5	3.82	0.42	0.47
c. —Thyroid, 2.5 mgm.....	0.3	0.0075	2.5	2.8

Summary. b. Potassium iodide containing 3.82 mgm. iodine had a very slight effect. c. Thyroid containing 0.0075 mgm. iodine (or 500 times less) protected against about 7 times the fatal dose. The iodine of the thyroid was about 3000 times as active as that of the potassium iodide.

SERIES XVII (AUGUST, 1908)(XI. 50)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MG. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.18	0.19
b. —Iodalia, 0.01 gm.....	1.05	0.105	0.28	0.30
c. —Potassium iodide, 5 mgm.....	76.5	3.82	0.22
d. —Sajodin, 0.02 gm.....	24.5	4.9	0.25	0.28
e. —Thyroid, 1 mgm.....	0.11	0.0011	0.9	1.0
f. —Thyroid, 1 mgm.....	0.2	0.002	1.7	1.8

Summary. b. Iodalia containing 0.105 mgm. iodine protected against $1\frac{1}{2}$ times the fatal dose of acetonitrile. c. Potassium iodide

containing 3.82 mgm. iodine protected against $1\frac{1}{10}$ times the fatal dose. d. Sajodin containing 4.9 mgm. iodine protected against $1\frac{3}{10}$ times the fatal dose. e. Thyroid containing 0.0011 mgm. iodine protected against $5\frac{1}{10}$ times the fatal dose. f. Thyroid containing 0.002 mgm. iodine protected against $9\frac{2}{5}$ times the fatal dose. Iodalia containing 0.105 mgm. iodine was more effective than potassium iodide and sajodin containing 35 to 47 times as much iodine. Thyroid containing nearly 100 times less iodine than iodalia protected against 3 times as much acetonitrile.

SERIES XVIII (APRIL, 1907)(VII. 148)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.45	0.50
b. —Potassium iodide, 10 mgm.....	76.5	7.6	0.7	0.75

Summary. Potassium iodide containing 7.6 mgm. iodine protected against $1\frac{1}{2}$ times the fatal dose of acetonitrile.

SERIES XIX (OCTOBER, 1905)(VI. 85)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.3	0.3
b. —Potassium iodide, 10 mgm.....	76.5	7.6	0.45	

Summary. Potassium iodide containing 7.6 mgm. iodine protected against $1\frac{1}{2}$ times the fatal dose of acetonitrile.

SERIES XX (MAY, 1908)(X. 171)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.33	0.36
b. —Potassium iodide, 10 mgm.....	76.5	7.6	0.95	1.1
c. —Thyroid, 1 mgm.....	0.129	0.00129	3.2	3.3

Summary. b. Potassium iodide containing 7.6 mgm. iodine protected against about 3 times the fatal dose of acetonitrile. c. Thyroid containing 0.00129 mgm. iodine protected against about 9 times the fatal dose.

SERIES XXI (APRIL, 1908) (x. 152)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONITRILE IN MG. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.4	0.42
b. —Potassium iodide, 10 mgm.....	76.5	7.6	0.54	0.56
c. —Thyroid, 1 mgm.....	0.084	0.00084	3.1	3.4
d. —Thyroid, 1 mgm.....	0.19	0.0019	4.6	4.8
e. —Iodothyrim (Bayer) (31), 5 mgm...	0.014	0.0007	1.9	2.0
f. —Iodothyrim (Bayer) (32), 5 mgm...	0.015	0.00075	0.58	0.7

Summary. b. Potassium iodide containing 7.6 mgm. iodine protected against $1\frac{1}{3}$ times the fatal dose of acetonitrile. c. Thyroid containing 0.00084 mgm. iodine protected against about 8 times the fatal dose. d. Thyroid containing 0.0019 mgm. iodine protected against more than 11 times the fatal dose (a smaller amount of thyroid would probably have produced as great an effect). e. One preparation of iodothyrim containing 0.0007 mgm. iodine protected against nearly 5 times the fatal dose of acetonitrile. f. Another preparation of iodothyrim containing slightly more iodine protected against only about $1\frac{1}{2}$ times the fatal dose. This is another illustration of the variability in strength of the commercial preparations of iodothyrim. It is worthy of notice, however, that the activity of even this weaker preparation of iodothyrim was, when reduced to terms of iodine content, thousands of times more active than potassium iodide.

SERIES XXII (JANUARY, 1908)(x. 86)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.5	0.55
b. —Iodoformogen, ¹⁵ 10 mgm.....	12.0	1.2	0.6	0.65
c. —Potassium iodide, 10 mgm.....	7.5	7.65	0.6	1.0
d. —Thyroid, 1 mgm.....	0.1	0.001	1.4	1.7
e. —Thyroid, 1 mgm.....	0.17	0.0017	2.7	3.0

Summary. b. Iodoformogen containing 1.2 mgm. iodine protected mice against about $1\frac{1}{5}$ times the fatal dose of acetonitrile. c. Potassium iodide containing 7.6 mgm. iodine also had a slight protecting action. d. Thyroid containing 0.001 mgm. iodine protected against about 3 times the fatal dose. e. Thyroid containing 0.0017 mgm. iodine protected against nearly 6 times the fatal dose.

SERIES XXIII (SEPTEMBER, 1905)(vi. 28)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.2	0.25
b. —Potassium iodide, 0.05 gm.....	76.5	38.2	0.25	0.35
c. —Thyroid, 0.05 gm.....	0.17	0.085	1.4	2.0

Summary. b. Potassium iodide containing 38.2 mgm. iodine had a slight effect. c. Thyroid containing 0.085 mgm. iodine protected against about 7 times the fatal dose. In both cases the doses were doubtless many times greater than was necessary to produce the maximum effect.

¹⁵ This is a mixture of iodoform and albumin (New and Non-Official Remedies, 1910, p. 110).

Several other experiments with large doses of potassium iodide were performed but the results were not essentially different from those above.

SERIES XXIV (FEBRUARY, 1909)(XI. 100)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.24	0.25
b. —Iodalia, 1 mgm.....	1.05	0.0105	0.28	0.3
c. — { (Iodalia, 1 mgm.....	1.05	3.0705	0.29	0.3
{ Potassium iodide, 4 mgm.....	76.5			
d. — { Iodalia, 0.5 mgm.....	1.05	2.9925	0.28	0.31
{ (Sajodin, 12 mgm.....	24.5			
e. —Potassium iodide, 4 mgm.....	76.5	3.06	0.26	0.27
f. —Sajodin, 12 mgm.....	24.5	2.94	0.29	0.3
g. — { Sajodin, 12 mgm.....	24.5	6.00	0.27	0.28
{ Potassium iodide, 4 mgm.....	76.5			
h. —Iodole, 1 mgm.....	88.96	0.8896	0.18	0.21
i. —Thyroid, 1 mgm.....	0.11	0.0011	1.8	2.6

Summary. Thyroid containing 0.0011 mgm. iodine protected mice against about 8 times the fatal dose of acetonitrile. Iodalia containing 0.0105 mgm. iodine and several of the other compounds and mixtures containing from about 3 to 6 mgm. iodine apparently had a slight effect. It seems evident, however, that the iodine of the thyroid was nearly 2000 times as active as that of the other iodine compounds (with the possible exception of the iodalia). The combinations of iodalia and potassium iodide, iodalia and sajodin and sajodin and potassium iodide although containing relative large amounts of iodine had no greater effect than these compounds singly.

SERIES XXV (SEPTEMBER, 1908) (XI. 58)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.31	0.32
b. —Hemol (iodo), 0.02 gm.....	16.00	3.2	0.37	0.38
c. —Iodosalicylic acid, 0.01 gm.....	73.8	7.38	0.65	0.75
d. —Sajodin, 1 mgm.	24.5	0.245	0.42	0.45
e. —Thyroid, 1 mgm.....	0.2	0.002	1.9	2.1

Summary. b. Hemol (iodo) containing 3.2 mgm. iodine protected mice against $2\frac{1}{4}$ times the fatal dose of acetonitrile. c. Iodosalicylic acid containing 7.38 mgm. iodine protected mice against $1\frac{1}{2}$ times the fatal dose of acetonitrile. d. Sajodin containing 0.245 mgm. iodine protected against about $1\frac{4}{10}$ times the fatal dose. e. Thyroid containing 0.002 mgm. iodine protected against $6\frac{2}{3}$ times the fatal dose. We may estimate the iodine of the thyroid as being about 600 times as active as that of the sajodin and several thousand times as active as that of hemol (iodo) and iodosalicylic acid.

SERIES XXVI (SEPTEMBER, 1908) (XI. 64)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls.....	0.11	0.13
b. —Thymol iodide, 0.01 gm.....	46.14	4.6	0.27	0.3
c. —Thyroid, 1 mgm.....	0.12	0.0012	1.6	1.7

Summary. b. Thymol iodide containing 4.6 mgm. iodine protected against at least twice the fatal dose of acetonitrile. c. Thyroid containing 0.0012 mgm. iodine protected against nearly 14 times the fatal dose.

SERIES XXVII (SEPTEMBER, 1905) (VI. 82)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. MOUSE	
			RECOVERED	DIED
a. —Controls	0.18	0.20
b. —Iodoform, 0.05 gm	96.7	48.3	0.35

Summary. Iodoform containing 48.3 mgm. iodine had apparently protected against about twice the fatal dose of acetonitrile. It should be added however that the mice which had received the iodoform lost nearly a third of their body weight; a similar loss of weight, caused by a restricted diet, caused a similar increased resistance.

SUMMARY OF EXPERIMENTS ON MICE.

The results of the above experiments are given in the following table. In preparing it all experiments in which an increase of resistance was produced were used. The range of amounts of iodine fed show the largest and smallest quantities, while the average is the mean of all the quantities in the particular group of experiments included. The average increase of resistance is the mean of the increases calculated for each experiment by dividing the mean of the "recovered" and "died" dose for the mice receiving the drug by the mean of the "recovered" and "died" dose of the controls. The figures are therefore the multiples of the dose which proved fatal to the controls in each series of experiments.

GENERAL SUMMARY OF EXPERIMENTS ON MICE

DRUG	NO. OF EXPERIMENTS	MGM. IODINE FED PER CAKE IN EACH EXPERIMENT.		AVERAGE INCREASE OF RESISTANCE TO ACETONITRILE
		RANGE OF AMOUNTS	AVERAGE AMOUNT	
Iodothyrene ¹⁶	4	0.0007 —0.00095	0.00078	4.76
Thyroid ¹⁷	8	0.00053—0.0011	0.00086	4.9
Thyroid.....	8	0.0012 —0.0018	0.0014	7.4
Thyroid.....	12	0.0019 —0.0038	0.0025	9.2
Thyroid.....	1	0.0194	21.0
Bladderwrack.....	3	0.0110 —0.0168	0.0137	1.5
Bladderwrack.....	11	0.0300 —0.2500	0.0871	1.4
Potassium iodide.....	3	0.76(?)—3.0	2.50	1.3
Potassium iodide.....	9	3.8 —7.6	6.34	1.4
Iodalia.....	2	0.0105	1.4
Iodalia.....	2	0.105 —1.05	0.527	1.7
Hemol-iodo.....	1	3.2	1.2
Iodalbin.....	2	2.1 —21.0	11.5	1.5
Iodoeosin.....	1	12.1	2.0
Iodoform.....	2	0.97(?)—19.34	19.34	1.5
Iodoformogen.....	1	1.2	1.2
Iodole.....	1	8.9	2.5
Iodonucleoid.....	1	46.0	1.0
Iodosalicylic acid.....	1	7.38	2.2
Sajodin.....	1	0.245	1.4
Sajodin.....	3	2.94 —4.9	3.59	1.4
Thymol iodide.....	1	4.6	2.4

The above table and protocols show:

1. *There was no parallelism between the amount of iodine administered and the physiological effect produced by any iodine compound except that of thyroid.* A glance through the above protocols suffices to show that with the exception of the thyroid

¹⁶Two experiments with iodothyrene of evidently inferior quality are omitted; the experiments with the other preparations show that iodothyrene is slightly less active than thyroid containing slightly more iodine.

¹⁷These experiments illustrate a point discussed in Bulletin 47 of the Hygienic Laboratory (p. 50), viz., that within certain limits there is a direct parallelism between the amount of iodine in thyroid and the increased resistance to acetonitrile, but that as the amounts of thyroid fed approach those giving the maximum effect this parallelism no longer holds; an increase in the amount fed no longer causes a corresponding increase in the degree of resistance.

there was within very wide limits no relation between the amount of the iodine compound fed and the physiological effect; in some cases a minute amount of a compound had a distinct effect and in others hundreds of times as much had no effect. The thyroid had under all circumstances a distinct effect.

2. *Minimum effective doses of the iodine compounds.* Some of the compounds used in the above experiments did not seem to offer much of interest in this connection and only a few experiments were performed with them. The following table gives the quantities of iodine contained in the minimum effective doses so far as these were determined. Two figures are frequently given as there was some uncertainty about the minimum dose.

	Mgms. iodine
Thyroid.....	0.00053
Extract of bladderwrack.....	0.01 to 0.017
Potassium iodide.....	0.76 to 2.0
Hemol-iodo.....	3.2
Iodalbin.....	2.1
Iodalia.....	0.01 to 0.1
Iodoeosin.....	12.0
Iodoform.....	0.97 to 19.34
Iodoformogen.....	1.2
Iodole.....	8.9
Iodonucleoid.....	46.0
Iodosalicylic acid.....	7.38
Sajodin.....	0.245 to 2.9
Thymoliodide.....	4.6

The results with thyroid, extract of bladderwrack, and potassium iodide have the most value for many experiments were performed with these compounds. These permit of the perfectly definite conclusion that in order to obtain a distinct degree of protection against acetonitrile it is necessary to give from 20 to 40 times as much iodine in the form of extract of bladderwrack as in the form of thyroid and from 80 to 200 times as much in the form of potassium iodide as in the form of bladderwrack.

The other compounds in the above table probably do not differ markedly from potassium iodide in this respect; only iodalia seems to belong rather to the class of bladderwrack but further experiments will be necessary to determine this point.

From these results we may conclude that an iodine compound producing a condition of increased resistance in mice to acetonitrile in doses containing less than 0.001 mgm. iodine per 4 grams cake (fed daily for a week or 10 days) is undoubtedly thyroid; an increase of resistance with doses between 0.01 and 0.001 indicates thyroid while a compound producing this effect in doses containing 0.01 to 1 mgm. iodine is probably bladderwrack.

3. *The maximum degree of resistance caused by iodine compounds.* The fatal dose of acetonitrile was not, in many cases, determined with sufficient accuracy to permit of positive statements as to the exact degree of resistance caused by the iodine compounds.

The following are as close approximations as possible; the figures represent the maximum increase of resistance expressed in multiples of the dose which proved just fatal to the controls.

Thyroid.....	13.7 (21 once)
Extract of bladderwrack.....	2.+
Potassium iodide.....	1.8 (3 in one case)
Hemol-iodo.....	2.2 (once)
Iodalbin.....	1.5
Iodalia.....	1.8
Iodoeosin.....	2.2 (once)
Iodoform.....	2.0
Iodoformogen.....	1.2
Iodole.....	1.5 (once)
Iodonucleoid.....	1.5
Iodosalicylic acid.....	1.2
Sajodin.....	1.6
Thymol iodide.....	2.4 (once)

The compounds fall into two classes—thyroid and all others. It is especially interesting to note that bladderwrack which is active in such minute amounts does not in large amounts cause a greater degree of resistance than do several other iodine compounds.¹⁸ These experiments suggest that if an iodine compound is found which in any dose causes a greater degree of protection than 2 or 3 times the fatal dose of acetonitrile it is probably thyroid.

¹⁸ We are not certain that the increased resistance in some of the above cases is to be attributed to a direct effect of the iodine compounds. Some of them caused a marked loss of weight, and a loss of weight is in some cases accompanied by an increased resistance.

4. *Relative activity of the above iodine compounds in terms of the iodine content.* The above figures enable us to estimate, roughly, the relative activity of the above compounds in their protecting action towards acetonitrile. Thus the minimum amounts of bladderwrack which had a distinct effect contained from 15 to 30 times as much iodine as the minimum amounts of thyroid used in the above experiments. The degree of protection afforded by these minimum amounts of thyroid was however from 3 to 9 times as great as that afforded by the minimum amounts of bladderwrack. Hence thyroid may be estimated as being from about 45 to 270 times as active as bladderwrack. When the experiments in which a direct comparison is possible are considered this figure is found to approximate 140.

A similar line of reasoning leads to the conclusion that bladderwrack is from 80 to 200 times as active as potassium iodide and the other iodine compounds (with the possible exception of iodalia) and that thyroid is from 10,000 to 40,000 times as active as the latter. The comparisons in the individual experiments support in general this conclusion.

A similar conclusion is arrived at when certain averages are considered. Thus the average amount of iodine in the potassium iodide of 3 experiments (in which excessive, evidently supermaximum doses were not given) was 2.5 mgms; that in 8 experiments with thyroid was 0.00086 mgm. The former amount was about 2900 times as great as the latter. The average protection afforded by the thyroid was 4.9; that by the potassium iodide 1.3. From these figures we may conclude if the activity be referred to the iodine that the iodine in thyroid is roughly 11,000 times as active as that in potassium iodide. All such calculations are, of course, but rough approximations but they serve to emphasize the fact that the activity of the iodine in thyroid (assuming that the iodine in proper combination is the most important active agent) is of an entirely different order from that of the iodine in potassium iodide.

Resistance caused by mixtures of iodine compounds. It is quite generally held that in certain cases greater physiological effects may be obtained by combining two or more drugs having a similar physiological action than can be obtained by the administration

of any one of them alone. Experiments with an anti-fat nostrum containing potassium iodide and bladderwrack suggested that a similar synergism may exist between these drugs, *i.e.*, that it may be possible to obtain a greater effect by giving two or more of them together than by either alone.

The above experiments show that in a number of cases the maximum degree of resistance could be increased by combining certain iodine compounds. The experiments may be tabulated as follows:

NO. OF SERIES		IODINE COMPOUNDS	MGM. IODINE PER CAKE	TOTAL IODINE IN MGM.	MULTIPLE OF FATAL DOSE FROM WHICH MICE RECOVERED
4.	c —	Potassium iodide 0.0093 mgm.....	0.007	0.014	1.4
		Bladderwrack 8.3 mgm.....	0.007		
	e —	Iodalbin, 0.0335 mgm.....	0.007	0.014	1.0
5.	d —	Bladderwrack 8.3 mgm.....	0.007	2.97	1.4
		Bladderwrack 0.1 gm.....	0.03		
	e —	Sajodin 0.012 gm.....	2.94	3.03	1.4
10.	c —	Bladderwrack 0.3 gm.....	0.09	3.8284	2.0
		Sajodin 0.012.....	2.94		
	e —	Bladderwrack 10.0 mgm.....	0.0084	1.06	2.1
13.	c —	Iodalbin 5.0 mgm.....	1.05	3.312	3.0
		Bladderwrack 10.0 mgm.....	0.0084		
	e —	Potassium iodide 5.0 mgm.....	3.82	1.782	2.0
	c —	Bladderwrack 0.3 gm.....	0.252	3.252	3.0
		Potassium iodide 2 mgm.....	1.53		
	d —	Bladderwrack 0.3 gm.....	0.252	3.312	3.0
	e —	Potassium iodide 4 mgm.....	3.06	3.192	3.5
		Bladderwrack 0.3 gm.....	0.252		
	f —	Potassium iodide 2 mgm.....	1.530	1.722	3+
	g —	Sajodin 6 mgm.....	1.370	3.00	1.5
		Bladderwrack 0.3 gm.....	0.252		
	i —	Sajodin 6 mgm.....	1.47	3.192	3.5
24.	c —	Potassium iodide 2 mgm.....	1.53	3.0705	1.2
		Sajodin 6 mgm.....	1.47		
	d —	Iodalia 1 mgm.....	0.0105	3.00	1.5
	e —	Potassium iodide 4 mgm.....	3.06	2.9925	1.2
		Iodalia 0.5 mgm.....	0.0525		
	f —	Sajodin 12 mgm.....	2.940	6.00	1.1
	g —	Sajodin 12 mgm.....	2.94	6.00	1.1
		Potassium iodide 4 mgm.....	3.06		

Thus in certain cases a mixture of bladderwrack and other iodine compounds produced a higher degree of resistance than could be obtained with bladderwrack or the other iodine compounds alone. The maximum protection obtained was $3\frac{1}{2}$ times the fatal dose. This was far less than the maximum effect obtained with thyroid and the amount of iodine in thyroid sufficient to produce an equally great resistance was thousands of times less.

These results make it necessary to add to the conclusion drawn above as to the significance of a degree of protection against acetonitrile greater than about twice the fatal dose; the latter, in the light of these results, may be due either to thyroid or to a mixture of iodine compounds containing bladderwrack. Inasmuch however, as such mixtures are active only when they contain a relatively very large amount of iodine there would probably be no difficulty in distinguishing between the iodine of thyroid and that of such mixtures.

b. *Experiments on Rats.* Thyroid and other iodine compounds, if they have any effect, diminish the resistance of rats to acetonitrile; the diminution from thyroid is roughly parallel to the iodine content of the latter.

SERIES I (OCTOBER, 1908) (XI. 82)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONITRILE IN MGM. PER GM. RAT	
			RECOVERED	DIED
a. Controls.....	3.0	3.3
b. Bladderwrack, 0.1 gm.....	0.084	0.084	3.3	...
c. Sajodin, 5 mgm.....	24.5	1.225	2.4	2.5
d. Thyroid, 1.0 mgm.....	0.18	0.0018	1.4	1.6

Summary. Bladderwrack containing 0.084 mgm. iodine had no effect upon the resistance of rats to acetonitrile. The fatal dose of acetonitrile for rats which had received 1.225 mgm. iodine in sajodin was about 0.8 of that fatal to the controls; that for rats which had received 0.0018 mgm. iodine in the form of thyroid about 0.5.

SERIES II (MAY, 1907) (VII. 94)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. RAT	
			RECOVERED	DIED
a. Controls.....	3.8	4.0
b. Bladderwrack, 0.2 gms.....	0.086	0.172	2.2	2.8
c. Potassium iodide, 0.02	76.5	15.30	2.0	2.4
d. Thyroid, 0.01 gm.....	0.18	0.018	...	2.0

Summary. The fatal dose of acetonitrile for rats which had received 0.172 mgm. iodine in the form of bladderwrack and 15.28 mgm. in the form of potassium iodide was about 0.6 of that for the controls. Thyroid with 0.018 mgm. had lowered the resistance still more. These results suggest that iodine in the form of bladderwrack is much more active than that of potassium iodide and that thyroid is more active than either.

SERIES III (NOVEMBER, 1908) (XI. 85)

DRUG	PER CENT IODINE IN DRUG	MGM. IODINE PER CAKE	FATAL DOSE OF ACETONI- TRILE IN MGM. PER GM. RAT	
			RECOVERED	DIED
a. Controls.....	3.2	3.4
b. Bladderwrack, 0.2 gm.....	0.03	0.06	2.7	2.8
c. Iodalia, 1 mgm.....	1.05	0.0105	2.8	3.3
d. Potassium iodide, 5 mgm.....	76.5	3.82	3.0	...
e. Sajodin, 1 mgm.....	24.5	0.245	...	2.4
f. Thyroid, 1 mgm.....	0.11	0.0011	1.2	1.5
g. Thyroid, 1 mgm.....	0.12	0.0012	1.7	2.0

Summary. Bladderwrack with 0.06 mgm. iodine lowered the resistance of rats to acetonitrile to about 0.8 of that of the controls. Sajodin with 0.245 mgm. lowered it to about 0.7. Thyroid with 0.0011 mgm. and 0.0012 mgm. lowered it to 0.4 and 0.58. Potassium iodide with 3.82 mgm. had little if any effect. These experiments suggest that bladderwrack and sajodin are more active than potassium iodide and that thyroid is much more active than any of the others.

SUMMARY OF EXPERIMENTS ON RATS

The amounts of iodine in the minimal amounts of the above compounds which caused any lowering of the resistance of rats to acetonitrile were as follows:

	AMOUNT OF IODINE (IN MGM.) IN MINIMUM ACTIVE DOSES
Thyroid.....	0.0011
Bladderwrack.....	0.06
Potassium iodide.....	15.28
Sajodin.....	0.245 (or 1.225)

The experiments show clearly that thyroid is in a class by itself in its effect upon the resistance of rats to acetonitrile; it required more than 50 times as much iodine in the form of bladderwrack to cause a lowering of resistance as it did for iodine in the form of thyroid. Bladderwrack seemed to be more active than potassium iodide or sajodin. An iodine compound which distinctly lowers the resistance of rats to acetonitrile in doses containing less than 0.06 mgm. iodine is probably thyroid.

The maximum effects upon the resistance of rats to acetonitrile was caused by thyroid as is shown by the following table:

	FATAL DOSE COMPARED TO THAT OF THE CONTROLS
Thyroid.....	0.4
Bladderwrack.....	0.6
Potassium iodide.....	0.6
Sajodin.....	0.7

CONCLUSIONS

a. *From experiments on mice.* 1. Thyroid differs from all other iodine compounds studied; (a) in that it causes a greater degree of resistance to acetonitrile; (b) in that it is active in much smaller amounts; (c) in that it is invariably active and (d) in that there is a parallelism between the physiological activity and the amount of iodine it contains.

2. From the facts (a) that with other iodine compounds it is impossible to obtain more than a slight degree of resistance; (b) that these are not invariably active and (c) that there is no parallelism between the amount of iodine or of the compound given

and the physiological effect it is concluded that the action of these compounds is secondary and dependent at least to a large extent upon their ability to supply iodine to the thyroid or to stimulate the latter in some way and that the ability of these compounds to influence the thyroid depends to a considerable extent upon the condition of the latter; that is, the degree of activity of these compounds is largely limited by the conditions of the thyroid. This hypothesis explains (a) why only a limited degree of resistance is obtained with these compounds, (b) why they are not invariably active and (c) why, within wide limits, there is no parallelism between the amount of iodine given and the physiological activity.¹⁹

Further support for the hypothesis that these iodine compounds influence the resistance of mice to acetonitrile through the thyroid was given in the introduction.

3. Bladderwrack differs from the other iodine compounds studied (except the thyroid) in that very much smaller amounts (in terms of iodine) are effective in protecting mice against acetonitrile; it was estimated above that the iodine of bladderwrack was as efficient as 80 to 200 times as much iodine in the form of potassium iodide. Bladderwrack differs from the thyroid in the same way as do the other iodine compounds studied; that is, (a) it does not invariably have an effect; (b) there is no parallelism between the effect and the amount of iodine; (c) the maximum protection afforded is slight as compared with that of the thyroid. Hence it is concluded that the action of bladderwrack is not a direct one upon the organism as is that of thyroid but an indirect one exerted through the thyroid gland and further that the reason it

¹⁹ In harmony with this conclusion is the statement of Kocher that in parenchymatous goiters very small doses of potassium iodide are as efficient as larger doses; *i.e.*, if the thyroid is in a condition to respond to iodine at all very small amounts suffice. See also Marine and Lenhart, *Archives of Internal Med.*, 1909, 4, p. 253.

As has already been stated one of the present writers has in press a paper in which the means by which the ability of the thyroid to respond to iodine may be influenced experimentally is discussed. In some conditions one iodine compound is more active than others. Thyreotropism is dependent on the condition of thyroid as well as on the character of the iodine compounds

is so much more active than other iodine compounds is that it has a specific affinity for the thyroid, or in other words, because it is "thyreotropic."

4. Combinations of several iodine compounds are as a rule more active than single compounds containing a larger amount of iodine. This is especially the case when bladderwrack is contained in the mixture.

b. *From the experiments on rats.* Conclusions similar to the above may be drawn from the experiments on rats; thyroid lowers the resistance of rats to acetonitrile in much smaller doses than do the other iodine compounds, and bladderwrack is effective in smaller amounts than are potassium iodide and sajodin.

SUMMARY

1. The iodine of bladderwrack has a specific thyreotropic action; it is from 80 to 200 times as active as that of any other iodine compound studied (with the exception of the thyroid).

2. It is possible to distinguish, by means of physiological tests, between the iodine of the thyroid and that of bladderwrack and between the latter and that of other iodine compounds.

3. It is possible to obtain more marked physiological effects with mixtures of iodine compounds than with the latter alone.

Note. Carlson and Woelfel state in the April number of the *Am. Jour. of Physiol.* (vol. 26, p. 32) "that the acetonitrile test is probably a test for iodine compounds in general rather than a test for specific thyroid secretions." That the test is not one for iodine compounds in general we consider to be conclusively established by the foregoing experiments; in fact we consider it sufficiently established by our previously published experiments in which it was shown that "iodine-free" thyroid (thyroid which when examined in amounts up to 1 gm. did not give even a qualitative test for iodine) protected mice against more than 10 times the fatal dose of acetonitrile whereas potassium iodide even in large doses had but a slight effect.

Carlson and Woelfel record some interesting results of an attempt to detect, by means of the acetonitrile test, thyroid secretion in the lymph coming from the thyroid. The results were entirely negative as we think was to be anticipated even assuming that the secretion reaches the circulation by this route. In the first place there is much evidence that the

amount of secretion necessary to maintain health in adults is very small: in fact many adult animals show no symptoms when the thyroids are removed suggesting that there is little if any secretion. Efforts have been made to calculate the amount of secretion necessary for human beings from the amount of thyroid which must be fed to cretins or myxoedematous patients in order to completely overcome the thyroid deficiency. From the fact that some of the most pronounced cases of myxoedema are kept in perfect health by the administration *per os* (by which some may be destroyed before absorption) of 1 or 2 grains (64 to 128 mgms.) of desiccated thyroid every 3 or 4 days it has been estimated that the normal daily secretion may not be greater than that contained in 20 or 30 mgms. of thyroid; that for an adult dog of average size may be estimated at about $\frac{1}{10}$ of this. Carlson and Woelfel state that they fed daily 1 cc. of lymph obtained from colloid and hyperplasia goiters; in another part of their paper they state that goiters weighing from 100 to 500 gms. secrete, without massage, from 20 to 30 cc. of lymph in 2 hours or from 240 to 360 cc. in a day. Thus they seem to have fed daily not more than $\frac{1}{200}$ of the lymph secreted; assuming on the basis of the above calculations that 2 or 3 mgms. of desiccated thyroid is roughly equivalent to the secretion of the thyroid of a dog, the authors could scarcely have fed more than the equivalent of $\frac{1}{20}$ to $\frac{1}{100}$ of a mgm. of dried thyroid daily.

With thyroid containing a high percentage of iodine we have obtained distinct results when 0.1 mgm. thyroid was fed; with thyroid containing a smaller percentage of iodine from 5 to 10 times as much was often necessary. In other words, in order to obtain clearly positive results, it is necessary to have from 50 to 100 times as much thyroid as could reasonably be expected to be present in the amount of lymph used by these authors. Since several of the thyroids from which the lymph was obtained contained little or no iodine, whatever secretion was contained in the lymph was probably very poor in iodine and iodine poor thyroid has a low degree of physiological activity.

Of course such calculations as the above have no value except as probably indicating with what order of quantities we are dealing, but they seem to us to show that even the negative results of Carlson and Woelfel throw little light on the question under investigation.

The authors state that they are determining whether removal of the thyroid in rats and mice alters the resistance of these animals to acetone-trile and state that so far uniformly negative results have been obtained. We reported such experiments upon guinea-pigs several years ago; the

results were negative as was to have been anticipated in animals in which removal of the thyroids usually has no discernible effect. We also doubt whether distinct results could be obtained in animals which do react in other ways to removal of the thyroid; the other changes occurring would probably obscure whatever changes in the resistance to acetonitrile might occur. Hence we can not agree that negative results of this character would make the acetonitrile test for thyroid secretion doubtful. We may add that we have never made positive claims for this reaction as a test for *thyroid secretion*; we claim that when properly and critically applied it is an extremely delicate test for thyroid gland (including iodothylin) and one by which this can be detected with more certainty than by any other method at present known. We do believe however, that there is considerable evidence, somewhat indirect it is true, that it is a test for thyroid secretion as the latter occurs in the body but that the method which we have pursued of increasing the activity of the gland by the administration of iodine is a more promising one than that adopted by Carlson and Woelfel.

In connection with the negative results obtained by Carlson and Woelfel in applying the acetonitrile test to the blood of a case of exophthalmic goitre it may be of interest to add that Dr. L. B. Wilson, in a personal communication, states that he tested the blood of four cases of exophthalmic goitre and obtained a positive reaction in each. In two of the cases the reaction was very marked; in the other two it was perfectly definite although less marked. Control experiments were made with the blood of normal individuals or with that of other patients.

ON INSUFFLATION OF THE LUNGS WITH HYDROGEN; WITH CARBON DIOXIDE; AND WITH AIR¹

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A few years ago, Gies and Meltzer (American Journal of Physiology, 1903, ix, 18-24) reported results on insufflation of the lungs with hydrogen gas in strychninized rabbits in which they found that strychnine spasms could be controlled for 31 minutes, at the end of which time the animal manifested no "signs of asphyxia, dyspnoæ, or cyanosis." Moreover, in the control animal without strychnine an insufflation with pure hydrogen might be continued for 18 minutes without asphyxia. As this was of interest in connection with observations on the control of strychnine spasms by carbon dioxide (American Journal of Physiology, 1908, xxii, 440), we determined to observe the results of insufflation for ourselves. In order to avoid the admixture of air with hydrogen or carbon dioxide, the gases (hydrogen, carbon dioxide and air) were stored in tanks under equal pressures and the tanks were so connected together and with the tube in the trachea, that a change from one tank to another could be made in a very few seconds. By alternate compression and release of the tube leading from the tanks and the side tube from the tracheal cannula, rhythmic insufflation of the lungs was readily effected and regulated. A water valve on the side, or expiratory tube prevented the possible entrance of air by that route. In the first series of experiments, cats were used. Carotid blood pressure was recorded with a mercury manometer and respiratory movements recorded by means of a lever resting

¹ Read by title, First Annual Meeting, Soc. of Pharm. and Exp. Ther., 1909.

on the thorax; and also with a tambour connected with the tracheal tube. In the second series, rabbits were used and a graphic record only of changes in the respiratory (intra-tracheal) pressure was made.

Two cats were employed in the first series of experiments and two rabbits in the second series. As the results were the same in all cases, only one protocol from each series will be given.

PROTOCOL I

May 8, 1909. Large grey adult male cat, No. 2.

3:48:30Etherized.
 3:56Pulse 23 in 10 seconds, respiration 10.5 in 10 seconds.
 3:57Pulse 30 in 10 seconds, respiration 11.
 4:00Pulse 30 in 10 seconds, respiration 9.
 4:03:30Pulse 30 in 10 seconds, respiration 11.
 4:05Good eye reflex. Both pupils dilated.
 4:05:30Discontinued ether.
 4:06Carotid blood pressure; intra-tracheal pressure recorded by tam-
 bour connected with side tube.
 4:06:30Pulse 23 in 10 seconds, respiration 8. Mean blood pressure 121. Cat
 struggling slightly.
 4:07Gave air from tank.
 4:08Pulse 28 in 10 seconds, respiration 8, blood pressure 123.
 4:08:15Changed from air to hydrogen, blood pressure 128.
 4:08:30Spasms. Respiration 6 in 10 seconds, blood pressure 88.
 4:09Animal stopped breathing.
 4:09:15Air from tank, blood pressure 32.
 4:09:30Blood pressure 100.
 4:09:45Powerful gasps.
 4:11Strong eye reflex.
 4:11:15Cat struggling. Good eye reflex. Pulse 23 in 10 seconds, respira-
 tion 11 in 10 seconds, blood pressure 100.
 4:11:20Turned on hydrogen. Blood pressure 105.
 4:11:40Blood pressure 115.
 4:12Cat struggles powerfully. Rigid asphyxial spasms, blood pressure
 53.
 4:12:15Relaxing. Stopped breathing. Pupils dilated. No respiratory
 movements. Completely quiescent and relaxed.
 4:13Changed to air, blood pressure 18.
 4:13:15Blood pressure 116.
 4:13:30Strong respiratory discharges.
 4:14:30No eye reflex.
 4:14:45Eye reflex present.
 4:15Pulse 22 in 10 seconds, respiration 11. Blood pressure 104; turned
 on hydrogen.

- 4:15:15.....Blood pressure 108.
 4:15:30.....Blood pressure 50.
 4:15:45.....Powerful respiratory efforts. Blood pressure falling.
 4:16:30.....Pulse 14 in 10 seconds, respiration 11. Gasp. Blood pressure 34.
 4:16:45.....Reflex of eye gone. Another gasp. Blood pressure 31.
 4:19:30.....Removed drum. Blood pressure 5.
 New drum, no. 2. Blood pressure 10.
 4:20:30.....Changed to air.
 4:22.....Removed respiratory tracing.
 4:22:30.....Opened thorax of cat.
 4:23:30.....Heart stopped. Blood pressure 7.
 4:23:45.....Massaged heart. Blood pressure 17. Lungs pink.
 4:25:30.....Cat dead (clinically). Only residual blood pressure.
 4:26:15.....Auricles pulsating feebly.
 4:36.....An analysis of a sample of hydrogen from the tank showed that the
 gas contained less than 0.4 per cent oxygen, and no carbon
 dioxide.

PROTOCOL II.

May 26, 1909. Adult white (albino) rabbit, No. 2.

- 11:33.....Etherized.
 11:39.....Respiration 19.
 11:42.....Respiration 17.
 11:42:45.....Turned on hydrogen.
 11:43.....Batting eyes. Pupils constricting.
 11:43:15.....Pupils strongly constricted.
 11:43:30.....Rabbit struggles strongly. Ears very blue. Pupils dilating.
 11:43:45.....Quiet. Discontinued hydrogen. No signs of life.
 11:44.....Began giving air.
 11:44:15.....Gasping.
 11:44:30.....Ears and tongue becoming pink. Animal recovering. Pupils very
 small.
 11:45.....Respiratory gasps.
 Discontinued air.
 11:45:15.....Normal respiration, 13 in 10. Struggling. Batting eyes. Pupils
 dilating.
 11:46.....Pupils dilated to maximum. Moving mouth, nose, etc.
 11:46:30.....Respiration 13.
 11:47.....Respiration 12.
 11:48.....Turned on carbon dioxide.
 11:48:15.....Powerful stimulating effect on respiration. Ears blue. Respiration
 almost stopped. Irregular.
 11:48:45.....Pupils dilating. Respiratory gasps. Ears and tongue very blue.
 11:49.....Gasping. Very blue.
 11:49:30.....Shaking tail. 6 gasps in 10 seconds.
 11:50.....Gasps ceased.
 11:50:15.....No sign of life. Turned on air.

11:51:30.....Beginning to gasp. Pupils becoming red. (Retinal glare.)
 11:51:45.....Ears and tongue pink. Animal in good condition.
 11:52:15.....Respiration 9 in 10.
 11:53:30.....Batting eyes.
 11:54:30.....Respiration 10 in 10 seconds.
 11:55:15.....Respiration 9 in 10 seconds; deeper.
 11:55:30.....Clamped trachea. Pupils constricted.
 11:56.....Winking eyes. Ears blue. Red glare (retinal) becoming blue.
 11:56:30.....Respiration 6 in 10 deep. Good eye reflex. Ear, mouth, etc., blue.
 11:56:45.....Respiration 5 in 10 seconds. General spasms.
 11:57.....Lid reflex active. Eyes protruding. Very strong spasms.
 11:58.....Pupils dilating. Still slight eye reflex present.
 11:58:15.....Pupils dilating fast. Respiration 2 in 10. Pupils widely dilated.
 Ears blue.
 11:58:45.....Expiratory standstill.
 11:59:30.....Heart beating feebly.
 12:00.....Gasp ceased. No evidences of life.
 12:00:15.....Artificial respiration. Pupils (glare) becoming red. Ears pink.
 12:00:45.....First gasp.
 12:01:15.....3 gasps in 10 seconds.
 12:01:30.....Pupils still dilated.
 12:02.....Eye reflex active.
 12:02:30.....Discontinued artificial respiration.
 12:03.....Pupils constricting. Respiration 6 in 10 seconds.
 12:03:15.....Pupils still constricting. Discontinued experiment.

RESULTS

With hydrogen gas containing less than one percent of oxygen, a typical asphyxial blood pressure curve was written by the mercury manometer, only the events (rise and fall of pressure and slowing of the heart) appeared sooner and were of shorter duration than was the case when asphyxia was produced by clamping the trachea. Also respiratory effort disappeared and death occurred more quickly than with simple clamping of the trachea.

If before the final stage of asphyxia was reached the hydrogen was shut off and air turned on, resuscitation rapidly occurred. With a mixture of hydrogen and air, a state of partial asphyxiation could be produced and indefinitely maintained. The same was true for a mixture of nitrogen and oxygen which was prepared by partially removing the oxygen from air. With carbon dioxide the results were essentially the same as with hydrogen, the signs of death only being somewhat more rapid.

Since the above was written, another series of experiments¹ has been performed with the view of obtaining data on the bellows factor.

The arrangement of the apparatus was such that the lungs of a cat could be insufflated with gas from a gasometer either by pumping through a bellows, or by compressing the gas in the gasometer and alternately compressing and releasing the tubes connected with the trachea.

Briefly summarized, the results were as follows:

1. Pulmonary insufflation with hydrogen *via* a dry bellows was followed by symptoms of only partial asphyxia.
2. After wetting (tightening) the bellows, similar insufflation was followed by symptoms of complete asphyxiation and death.
3. Insufflation directly from the gasometer with compressed hydrogen gave symptoms of rapid and complete asphyxia; but after all evidence of discharge from the respiratory center had disappeared and the blood pressure had been reduced to 24 mm. mercury and while it was still rapidly falling, insufflation with air quickly resuscitated the animal.
4. Using hydrogen containing a little air, it seemed that insufflation either with or without the bellows led to symptoms of acute and complete asphyxia when the pulmonary ventilation was not greater than adequate ventilation with air; but when much greater, asphyxial symptoms were less pronounced.

CONCLUSION

1. Ventilation of the lungs with hydrogen containing little air is followed by symptoms of asphyxia.
2. If no oxygen be present, death will rapidly ensue with any rate or method of ventilation.
3. If hydrogen containing a small percentage of oxygen be employed, death will rapidly result when the rate of ventilation is as great as in just adequate artificial respiration with air. Death under such oxygen-hydrogen insufflation seems at least to be delayed by increasing the ventilation rate.
4. Similar results follow the use of imperfectly air-tight bellows. For example, with a pulmonary ventilation much greater than that adequate with air, if the insufflation be carried out by compression of the gas, death rapidly occurs; but if the gas be pumped through a leaky bellows, a ventilation not much greater than that adequate with air may not immediately be followed by death or even the severer grades of asphyxial

¹ Received for publication, April 15, 1910.

symptoms. If now the bellows be treated to a tightening process (*e.g.*, wetting), death may soon occur when the experiment is again performed.

Under the conditions of our experiments, we therefore conclude that insufflation of the lungs of cats or rabbits with hydrogen gas leads rapidly to cessation of respiratory and circulatory movements (clinical death). Also, if not delayed too long, resuscitation may be accomplished by insufflating the lungs with air, accompanied by cardiac massage if the circulation has fallen very low.

In conclusion, we may say that in general our results are in agreement with those of Heidenhain and Krause, Thiry, and Rosenthal (See Gies and Meltzer, *American Journal of Physiology*, *loc. cit.* for literature).

THE INFLUENCE OF INTRAVENOUS INJECTIONS OF SPARTEINE AND ADRENALIN ON THE HEART OF THE DOG

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Fleisher and Loeb¹ have shown that a single injection of adrenalin, preceded by an injection of either sparteine sulphate or sodium caffeine benzoate, will produce in rabbits a myocarditic lesion, visible to the naked eye. Such gross myocarditic lesions were noted in 60 per cent of the rabbits injected with adrenalin and sparteine or caffeine, changes in the myocardium were noted on microscopic examination in approximately 90 per cent.

It was possible by this rather simple method to produce experimental heart lesions in rabbits and thus an easily accessible method had been devised of testing experimentally the influence of myocarditic lesions upon other processes. It appeared of interest to learn whether similar myocarditic lesions could be produced in other animals and at the suggestion of Dr. Loeb we have determined the influence of injections of sparteine sulphate and adrenalin on the production of myocarditic lesions in dogs.

Since the jugular vein of the dog is easily accessible, we injected the various drugs into this vein. All the operative procedure was carried out with the usual aseptic precautions.

Different quantities of sparteine sulphate, varying from 0.075 gm. to 0.03 gm., were injected into dogs; generally either 0.050 gm. or 0.03 gm. of sparteine sulphate were injected into each dog. As a rule after the injection of the sparteine four minutes were allowed to elapse before the adrenalin was injected. One cubic

¹ Archives of Int. Med. 1909, vol. iii, 78.

centimeter of this latter substance was usually injected; however, as much as 2 cc. were injected in some cases and as little as 0.8 cc. in other cases.

Eighteen dogs were used in this series of experiments; three of these dogs died shortly after the injection of the sparteine sulphate and before the injection of adrenalin, so that the hearts of fifteen dogs were available for the purpose of studying any changes in the myocardium. These fifteen dogs which survived the injection of sparteine and adrenalin were examined at various periods after injection.

Six of these dogs were examined one week after the injection. At this period after the injection of sparteine and adrenalin, rabbits showed a gross myocarditic lesion. Not one of the six dogs examined at this period showed any changes in the heart. Neither paleness, hardness nor thickening of the cardiac musculature were noticeable.

One dog examined forty-seven days after the injection of sparteine and adrenalin showed no change which was visible to the naked eye, either on the pericardial surface or in the transverse section of the heart wall.

The eight other dogs were examined at various intervals, the last dog being killed 58 days after the injection. Not one of the hearts of these dogs showed a microscopic lesion.

Hearts of six of the dogs were examined microscopically; sections of the tissue from the left ventricle close to the base (the usual site of the myocarditic lesion in rabbits), from the septum and from the right ventricle were examined. In none of these were any appearances noted which suggested ether degenerative changes of the muscle fibers or an increase of connective tissue such as had been found in the hearts of rabbits injected with sparteine and adrenalin.

Besides the hearts, the aortas of these dogs were also examined; in no case could arteriosclerotic lesions be found.

The intravenous injection of sparteine and adrenalin into these dogs produced approximately the same symptoms that such injections had produced in rabbits. Thus following the injection of sparteine into dogs the respiration was slowed and became irreg-

ular, the heart beat became a little weaker. The heart beat soon became again normal but the respiration continued to be slow and weak. When the adrenalin was injected the heart beat was slowed for a few minutes, then became rapid, irregular and somewhat weak. The respiration was deep and slow but it gradually became normal. The heart became more regular and about 10 to 15 minutes after the injection it was again normal. Whether the animals showed weakness as a result of the injection of sparteine and adrenalin it was impossible to state since the administration of ether during the preceding operation caused weakness.

It appears, therefore, that the intravenous injection of sparteine sulphate followed by the intravenous injection of adrenalin does not produce myocarditic lesions in dogs, a fact which seems to us to be of great interest, inasmuch as the lesions produced by similar injections in rabbits are so very pronounced. It is probable that this difference is due to the fact that the heart of the dog is relatively stronger than that of the rabbit and is consequently better able to resist the injurious effect of these substances. The results of these experiments with dogs, therefore, confirm the explanation which Fleisher and Loeb offer for the causation of myocarditic lesion in rabbits—namely, that they are due to excessive mechanical strain.

IN REGARD TO THE DETOXIFICATION OF BENZOIC ACID BY OPTICAL ISOMERS OF LEUCIN

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By an experimental study of the action of certain drugs,¹ it has been shown that the animal organism is capable of distinguishing between stereoisomeric substances. It has been found that the stereoisomers of such chemical compounds as tartaric acid² β -oxybutyric acid,³ carbohydrates⁴ and amino acids meet qualitatively or quantitatively with a different fate in their passage through the animal organism. Thus the question of chemical space relation is of the greatest significance both to physiological chemistry and to pharmacology.

2. It is possible that the organism may alter its behavior toward stereoisomeric substances under varied physiological conditions and our experiments were undertaken primarily to throw some light upon this question.

Certain amounts of the monamino acids which are obtained by hydrolysis of the proteins, that is to say natural amino acids, are altered in some way or other when administered to the animal organism, for

¹ The literature concerning the action of the optical isomers of certain drugs on the organism is given in the paper of Grove. On the toxicity of dextro, laevo and inactive camphor. *J. Pharm. and Ex. Thera.* 1910, i., p. 445.

² Brion: Ueber die Oxydation der stereoisomeren Weinsäuren im tierischen Organismus. *Zeitschr. f. physiol. Chem.*, 1898, 25, p. 283.

³ McKenzie: The resolution of β -hydroxybutyric acid into its optically active components. *The Chem. Soc.*, 1901, 81, part ii, p. 1409.

⁴ Neuberg und Wohlgemuth: Ueber das Verhalten stereoisomerer Substanzen im Tierkörper (Arabinosen). *Zeitschr. f. physiol. Chem.*, 1902, 35, p. 41.

they are not excreted as such in the urine. This fact prompted a number of investigators to study the excretion of the amino acids under various physiological and pathological conditions. An estimation of these substances in the urine was made both before and after their administration. Thus Glässner⁵ fed certain amounts of glycoll, alanin, leucin and asparagin to normal individuals and to patients suffering with various diseases for the purpose of testing the function of the liver. In certain diseases associated with destructive processes in the parenchyma of the liver (syphilis, fatty degeneration, cirrhosis and phosphoric acid poisoning) a part of the administered acids passed the organism unchanged.

The behavior of the stereoisomers of the monamino acids is however of greater importance in connection with the subject under discussion.

Aberhalden and Kautzsch⁶ are of the opinion that it may be of great value to determine the quantities of racemic amino acids which the organism can decompose under normal and pathological conditions. Such investigations would furnish a new method for testing the functional capacity of the organism. The most important result which was brought to light by the study of the fate of these stereoisomeric compounds was that those components of the amino acids which occur in nature are decomposed, while their optical antimers are excreted in the urine. It is of no consequence whether the substances are administered in their active form or as racemic compounds.

In the larger number of the experiments and particularly those upon man, the acids were given per os and the variations in the results noted by different authors are probably attributable, at least in part, to the changes, which these substances undergo in the intestinal tract and these changes may be different in different individuals. For instance Embden⁷ found more laevo alanin in the urine after feeding racemic

⁵ Glässner: Funktionelle Pruefung der normalen und pathologischen Leber. Zeitschr. f. experim. Pathol. u. Therap., 1907, 4, p. 336 (literature).

⁶ Abderhalden und Kautzsch, Der Abbau des d,l-Leucylglycins, etc. Zeitschr. f. physiol. Chem., 1906, 48, p. 557.

⁷ Embden: Die Aminosäuren im Harn. Verhandlungen des Congresses f. innere Medicin. Wiesbaden, 1905, p. 304.

alanin than did Brugsch and Schittenhelm.⁸ McKenzie,⁹ referring to the article of Brion,¹⁰ called attention to the possible influence of intestinal bacterial action and Neuberg and Wohlgemuth¹¹ who studied the fate of the mannoses insist that safe conclusions can only be drawn after the parenteral introduction of these substances. Furthermore the variations in the results of different authors may in part be due to the lack of uniformity in the analytical methods used. In the experiments reported by Embden¹² amounts of racemic alanin were given per os to man varying from 4–50 gm. After giving 50 gm. of this substance 18 gm. of approximately pure β -naphtalin sulfo alanin were isolated from the urine of the next six hours; after giving 11 gm. the urine contained 4.5 gm. of this rather impure compound and even after giving 4 gm. the transition of alanin in the urine could be proved. The naphtalin sulfo compound was found dextrorotatory and Bergell pointed out in the discussion that it was a mixture of β -naphtalin sulfo laevo alanin and the naphthalin sulfo compound of the racemic alanin given. Brugsch and Schittenhelm¹³ gave a gouty patient 15 gm. of racemic alanin and only a small part of it appeared in the urine as laevo alanin. The healthy individual excreted only 1.45 per cent as laevo alanin after receiving 35 gm. of racemic alanin. The relatively insignificant excretion of this stereoisomer foreign to the body justly caused surprise. Wohlgemuth¹⁴ feeding 35 gm. of racemic alanin to a gouty individual recovered a part of the alanin from the urine as the laevo modification. These authors did not find any marked differences between the normal and the gouty individual.

Rahel Hirsch¹⁵ giving racemic alanin to dogs, per os and subcutaneously, found that the starving animal excreted laevo alanin after doses of

⁸ Brugsch und Schittenhelm: Zur Stoffwechselfathologie der Gicht. V. Ueber den Abbau von Glycoll und Alanin, etc. Zeitschr. f. experim. Pathol. u. Therap., 1907, 4, p. 538.

⁹ McKenzie: *loc. cit.*

¹⁰ Brion: *loc. cit.*

¹¹ Neuberg und Wohlgemuth: Ueber das Verhalten stereoisomerer Substanzen im Tierkörper (Mannosen). Zeitschr. f. physiol. Chem., 1902–3, 37, p. 530.

¹² Embden: *loc. cit.*

¹³ Brugsch und Schittenhelm: *loc. cit.*

¹⁴ Wohlgemuth: Ueber den Aminosäurenstoffwechsel bei der Gicht. Zeitschr. f. Biochem, 1906, I. p. 332.

¹⁵ Hirsch: Ueber das Verhalten der Monoaminosäuren im hungernden Organismus. Zeitschr. f. experim. Pathol. u. Therap., 1905, I. p. 141. Zum Verhalten von Monoaminosäuren, etc. Zeitschr. f. experim. Pathol. u. Therap., 1906, 2. p. 668.

15 gm. while the normal animal destroyed this amount completely. A well fed dog deprived of its pancreas oxidized 15 gm. of racemic alanin while a starving dog, which had received phloridzin excreted laevo alanin after a dose of 10 gm. of racemic alanin. Brugsch and Hirsch¹⁶ substantiated these results by feeding racemic alanin to a female "hunger artist" and to a normal woman in 10 gm. doses. The "hunger artist" excreted laevo alanin while no appreciable quantities of alanin could be found in the urine of the normal individual.

These results are of the greatest interest since they suggest that the organism may be capable of changing its behavior to the stereoisomers of monamino acids under changed conditions. Unfortunately they have not remained without opposition. In the experiments of Oppenheimer¹⁷ who fed racemic alanin in doses of 10 gm. to well nourished individuals, in those of Plaut and Reese¹⁸ who fed 5 and 10 gm. racemic alanin to well nourished dogs, and of Schittenhelm and Katzenstein¹⁹ who fed 20 gm. of racemic alanin on several days to a normal dog in nitrogenous equilibrium, alanin was recovered from the urine and in the latter experiment in the laevo form. It may be mentioned that Abderhalden²⁰ found laevo alanin in the urine of a healthy individual after giving 10 gm. of racemic alanin, while in another case laevo alanin was found only in traces after the administration of 15 gm. of racemic alanin.

Abderhalden and Schittenhelm²¹ fed a dog in nitrogenous equilibrium with 10 gm. dextro alanin and 10, 15 and 20 gm. racemic alanin. The dextro alanin was completely destroyed. If however the dogs were

¹⁶ Brugsch und Hirsch: Gesamt N- und Aminosäurenausscheidung im Hunger. Zeitschr. f. experim. Pathol. u. Therap., 1901, 3, p. 638. Ueber die Ausscheidung von Alanin durch den Harn. Zeitschr. f. experim. Pathol. u. Therap., 1907, 4, p. 947.

¹⁷ Oppenheimer: Ueber die Ausscheidung von Alanin durch den Harn. Beiträge z. chem. Physiol. u. Pathol., 1907, 10, p. 273.

¹⁸ Plaut und Reese: Ueber das Verhalten in den Tierkörper eingeführter Aminosäuren. Beiträge z. chem. Physiol. u. Pathol., 1906, 7, p. 425.

¹⁹ Schittenhelm und Katzenstein: Verfütterung von i-Alanin im normalen Hunde. Zeitschr. f. experim. Pathol. u. Therap., 1906, 2, p. 560.

²⁰ Abderhalden: Ueber den Abbau von 2.5-Diketopiperazinen im Organismus des Kaninchens. Zeitschr. f. Physiol. Chem., 1908, 55, p. 384.

²¹ Abderhalden und Schittenhelm: Studien über den Abbau racemischer Aminosäuren im Organismus des Hundes unter verschiedenen Bedingungen. Zeitschr. f. physiol. Chem., 1907, 51, p. 323.

simultaneously fed with thyroid tablets the presence of dextro alanin in the urine could be demonstrated only after the larger doses of the racemic alanin.

All the experiments bearing upon this point showed that the organism is at least capable of decomposing the dextro alanin, that is the stereoisomer occurring in nature, to a much greater extent than the laevo alanin. Bergell und Blumenthal²² feeding and injecting 10–15 gm. of racemic alanin in normal individuals and in several patients (severe anemia, moribund patient, diabetes) could not recover any considerable amount of alanin from the urine. One of their experiments is of great interest. A patient in diabetic coma received 15 gm. of racemic alanin and excreted dextro alanin. A confirmation of this result would be of the utmost importance since it would show that the organism may under certain conditions reverse its behavior toward optical isomers.

Of the experiments made with other amino acids only a few need be mentioned. Wohlgemuth²³ administered racemic tyrosin, asparagin, glutaminic acid and leucin to rabbits per os, subcutaneously and intravenously with uniform results. He reports only one experiment with each substance; that is where the acids were given per os. The isomer occurring in nature was decomposed entirely or to a great extent while the isomer foreign to the body passed the organism unchanged. As an example; after a dose of 10 gm. of racemic leucin 2.5 gm. of dextro leucin were recovered from the urine, while the laevo leucin was found completely decomposed. Abderhalden and Samuely²⁴ found that only a part of racemic leucin is decomposed in the organism of the rabbit. On feeding 10 and 15 gm. they recovered from the urine 50–60 per cent of the calculated amount of dextro leucin. Upon feeding to dogs or injecting subcutaneously 10 gm. the excretion of leucin was doubtful.

²² Bergell und Blumenthal: Ueber einen neuen Befund beim Eiweissabbau des Diabetikers. *Zeitschr. f. experim. Pathol. u. Therap.*, 1906, 2, p. 413.

²³ Wohlgemuth: Ueber das Verhalten stereoisomerer Substanzen im thierischen Organismus. Die inactiven Monoaminosäuren. *Berichte d. deutsch. chem. Gesellsch.*, 1905, 38, p. 2064.

²⁴ Abderhalden und Samuely: Der Abbau des Leucins und des Leucyl-leucins im Organismus des Hundes. *Zeitschr. f. physiol. Chem.*, 1906, 47, p. 364.

Abderhalden and Kautzsch²⁵ upon the basis of further experiments, stated that a strong male rabbit did not excrete any dextro leucin when the quantity of the racemic acid given remained below 6 gm. Abderhalden and Wacker²⁶ were able to demonstrate the presence of dextro leucin in the urine of rabbits after the administration of 10 gm. racemic leucylglycin anhydride.

By a different method of experimentation Embden²⁷ succeeded in establishing another difference in the behavior of the dextro and laevo leucin. Perfusing the livers of dogs with certain amounts of laevo, dextro and racemic leucin it was found that the dextro and racemic leucin increased the formation of aceto acetic acid and acetone, while *under the same conditions* the laevo leucin did not have this effect. The experiments of Embden and Michaud²⁸ showed that the organs of animals are capable of decomposing the acetone bodies and their results may serve to explain why a certain amount of the dextro leucin escapes excretion as such.

Our experiments were undertaken to see if the organism would behave differently toward the stereoisomers of a monamino acid under abnormal conditions and we chose for this study rabbits poisoned with benzoic acid. In these experiments the rabbits received an undoubtedly fatal²⁹ dose of benzoic acid *i.e.*, 2 gm. per kilogram as sodium benzoate per os in a 4 per cent aqueous solution. In Wiener's experiments the fatal dose for rabbits was 1.7 gm. benzoic acid per kilogram, but one animal survived a dose of 1.99 gm. per kilogram. Our control animals, ten in number, died within twenty-four hours after the administration of the benzoic acid with exception of one white rabbit of 1.7 kilogram which died on the third day. To insure a uniform mode of nutri-

²⁵ Abderhalden und Kautzsch: *loc. cit.*

²⁶ Abderhalden und Wacker: Ueber den Abbau von 2.5-Diketopiperazinen im Organismus des Kaninchens. *Zeitschr. f. physiol. Chem.*, 1908, *57*, p. 325.

²⁷ Embden: Ueber das Verhalten der optisch-isomeren Leucine in der Leber. *Beiträge z. chem. Physiol. u. Pathol.*, 1908, *11*, p. 348.

²⁸ Embden und Michaud: Ueber den Abbau der Acetessigsäure im Tierkörper. *Ibid.*, 1908, *11*, p. 332.

²⁹ Kobert: Zur Kenntniss der Wirkung der Benzoesäure. *Schmidt's Jahrbuecher der ges. Medicin.*, 1888, *185*, p. 12.

tion the animals were kept on oats for several days before the experiments. Wiener³⁰ states that the rabbit survives after receiving a fatal dose of benzoic acid per os preceded by a subcutaneous injection of 2.62 gm. leucin (from protein) per kilogram. Cohn³¹ contested this statement and we thought it possible that our experiments might throw some light upon this controversy.

In our first experiments the rabbits were subcutaneously injected with synthetic racemic leucin (Kahlbaum) followed by benzoic acid per os.

Rabbit 1. 1.6 kg. It was intended to give the leucin (2.6 gm. per kilogram) in an aqueous suspension. Owing to the mechanical difficulties a considerable amount was lost. The animal became sick, the feces were semifluid, the fur bristling etc. After 24 hours it began to eat a little and improved generally. After 72 hours it became fairly lively, the feces appeared normal and it took its food well. Recovered.

Rabbit 2. 1.6 kg. The suspended leucin was filtered, the filtrate injected subcutaneously and the wet leucin was packed under the skin of the back after making a small incision. The loss of leucin was insignificant. The dose was 2.6 gm. leucin per kilogram. The rest of the day the animal was very quiet and refused food for some time, there was very little diarrhoea. The next day the animal was still rather quiet, its fur was in good condition and it took its food well; no diarrhoea. The following day the rabbit appeared normal. Recovered.

From these two experiments it appeared that leucin really possesses a detoxifying effect on the action of benzoic acid. Furthermore if we take into consideration the negative results of Cohn who used leucin in practically the same doses but derived from the decomposition of protein (*i.e.*, the laevo form) it seemed probable that the organism of the rabbit utilizes either both forms of leucin for the detoxification of benzoic acid or even prefers the dextro leucin for this purpose. We therefore tried to find the

³⁰ Wiener: Ueber das Glycocol als intermediaeres Stoffwechselproduct. Arch. f. experim. Pathol. u. Pharmacol., 1898, 40, p. 313.

³¹ Cohn: Zur Frage der Glykokollbildung aus Leucin im tierischen Organismus. Arch. f. experim. Pathol. u. Pharmacol., 1902, 48, p. 177.

minimal dose of racemic leucin necessary for this detoxification. The result may be seen in the following table.

RABBIT NO.	RACEMIC LEUCIN IN GRAMS PER KG.	RESULT	REMARKS
3	1.3	Died within 24 hrs.	Numerous small abscesses in liver.
4	1.5	Died within 24 hrs.	
5	1.75	Died within 24 hrs.	
6	1.75	Died within 24 hrs.	
7	2.0	Died within 24 hrs.	
8	2.0	Died within second 24 hrs.	Urine of fourth day free from albumen. No blood. Mortification at place of injection.
9	2.0	Sick, began to improve on second day. Recovered.	

In these experiments the leucin was injected in solution as sodium salt. This solution was rather strongly alkaline and as with subcutaneous injections of sodium sulphite a peculiar jelly-like infiltration occurred in the subcutaneous tissue which at times would spread considerably. In those animals which survived there appeared after a few days a slowly progressing mummification of the skin, which apparently did not interfere with the general welfare of the animals.

From the above results we believed that we were approaching the minimal detoxifying dose. But in three more experiments (rabbits nos. 10, 11, and 12) 2.63 gm. of leucin per kilogram were injected as sodium salt and all three animals died within the first 24 hours. In the case of two of these animals the gelatinous infiltration had spread widely to the dependent parts of the abdomen, the injections having been made under the skin of the back. It was thought possible that these changes may have interfered with the absorption of the leucin and for this reason several experiments were made in which leucin was given suspended in physiological salt solution through a small incision in the back. Later the place of the injection was laid open and the leucin which was

not absorbed was scraped out carefully, dried and weighed. The results appear in the following table.

RABBIT NO.	WEIGHT	TOTAL AMOUNT OF LEUCIN IN GRAMS	LEUCIN RECOVERED IN GRAMS	LEUCIN ABSORBED IN GRAMS PER KG.	RESULT	REMARKS
13	1600	4.2	1.6	1.6	Died after 60 hours	Abortion
14	1700	2.24	0.87	0.8	Died within 24 hrs.	
15	1450	3.81	1.28	1.74	Died within second 24 hrs.	Omentum studded with pearl-like masses of gelatin- ous consistency.
16	1420	3.73	0	2.63	Sick, recovered	No leucin recovered, killed on sixth day.
17	1240	6.45	3.53	2.35	Died within 24 hrs.	
18	1400	7.28	4.31	2.12	Died within second 24 hrs.	
19	1530	8.0	4.95	2.0	Died within 24 hrs.	

It can be seen that this method of administration does not insure a uniform absorption of the leucin.

Two rabbits (nos. 20 and 21) now received laevo leucin in solution in doses of 2.63 gm. per kilogram. This leucin was obtained from an acid hydrolysis of placenta and was prepared by the ester method. One of the animals died within the first, the other within the second 24 hours. Two other rabbits (nos. 22 and 23) received the same dose of synthetic laevo leucin ($[\alpha]_D^{20} = +14.6^\circ$ in 20% HCl) and these animals died within the next twenty-four hours.

It may be remarked that the laevo leucin derived from the protein was more easily soluble than the synthetic leucin. In one experiment (no. 20) 5.13 gm. of this amino acid and in the other (no. 21) 4.92 gm. were dissolved in 150 cc. of boiling physiological salt solution and it was found possible to inject the warm solutions before any of the leucin had separated. In experiment no. 22, 3.34 gm. of synthetic laevo leucin were dissolved in 130 cc. boiling saline. The leucin began to crystallize before the injection could be finished. The rest had to be redissolved

after addition of about 30 cc. of saline. In experiment, no. 23, 5.44 gm. of leucin were dissolved in 150 cc. of boiling saline with addition of 4.0 cc. 2N NaOH, but it crystallized too rapidly from this solution on cooling. It was redissolved making the solution up to 200 cc. and could then be injected warm before any crystallization occurred. In none of the animals was any leucin left at the points of injection. The two rabbits injected with the synthetic laevo leucin showed some gelatinous infiltration like that mentioned above. In the first this was insignificant, in the second, where some alkali had been added, it was more pronounced.

The following animals survived the benzoic acid poisoning after having received leucin.

NO,	SYNTHETIC RACEMIC LEUCIN IN GRAMS PER KILOGRAM	GIVEN
1	.1	In suspension
2	2.6	In suspension
9	2.0	In solution as sodium salt
16	2.63	In suspension

A number of animals received and absorbed leucin in doses of 2 gm. per kilogram and over without evidence of a detoxifying action.

NO,	SYNTHETIC RACEMIC LEUCIN IN GRAMS PER KILOGRAM	GIVEN
7	2.0	In solution as sodium salt
8	2.0	In solution as sodium salt
10	2.63	In solution as sodium salt
11	2.63	In solution as sodium salt
12	2.63	In solution as sodium salt
17	2.35	In suspension
18	2.12	In suspension
19	2.0	In suspension

Four rabbits (nos. 20, 21, 22, 23) received laevo leucin in solution in doses of 2.63 gm. per kilogram and none of these animals survived.

Our experiments with laevo leucin do not confirm the statement of Wiener. The possibility of a detoxifying action of the racemic leucin cannot be denied since of 12 animals absorbing 2 gm. leucin or more per kilogram four survived. At present we are unable to assign a reason for the lack of uniformity in our results. It is possible that the rate of absorption enters into consideration. Meissner and Shepard³² state that the formation of hippuric acid in rabbits may be dependent on the temperature of the surroundings so that at lower temperature more hippuric acid is excreted than at higher. This factor cannot have played any role in our experiments.

It remains to be noted that the postmortem examination of the rabbits did not, as a rule, show very intense lesions of the gastrointestinal tract. The stomach mucosa as well as that of the intestines was the seat of a more or less pronounced inflammation and sometimes hemorrhages were found. The lungs showed different degrees of oedema and in most instances hemorrhages of varying size. The viscera were rather rich in blood. Evidences of a coccidial infection were hardly ever absent. The urine always reduced Fehling's solution very strongly and this property was very likely due to the presence of benzoyl glycuronic acid which has been isolated by Magnus Levy.³³

It is evident that the detoxifying action of leucin on benzoic acid cannot be utilized for the solution of the question whether the organism of the rabbit behaves differently to the optical isomers of leucin under altered conditions. Therefore the urine of several animals was examined for the presence of dextro leucin after the administration of the racemic compound. The urine of rabbits nos. 13, 14, 15 and 16 was acidified strongly with hydrochloric acid, evaporated in vacuo at 40 degrees and extracted with ether. The residue was then extracted with absolute alcohol and this alcoholic extract evaporated to dryness in vacuo. The dry residue

³² Meissner und Shepard: Untersuchungen über das Entstehen der Hippursäure im tierischen Organismus. Hannover, 1866.

³³ Magnus Levy: Ueber das Auftreten einer Benzoessäure-Glycuronsäure-Verbindung im Hammelharn nach Benzoessäure Fütterung. Zeitschr. f. Biochem., 1907, 6, p. 502.

was taken up in absolute alcohol and esterified in the usual manner with gaseous hydrochloric acid. The esters were freed by means of sodium ethylate and distilled at 12 mm. pressure (Hg.) into sulphuric acid of known strength ($\frac{N}{5}$). The esters were hydrolyzed by boiling the sulphuric acid solution. The sulphuric acid was removed quantitatively with barium hydroxide. The filtered solution was evaporated on the water bath. The resulting leucin weighed 0.162 gm. and it gave the following rotation $[\alpha]_D^{20}$ in 20 per cent hydrochloric acid = $-9.7^\circ (+0.4^\circ)$. Two rabbits nos. 24 and 25, had received 2.63 gm. of racemic leucin in solution per kilogram. followed by 1.5 gm. benzoic acid per kilogram; No. 25 died within the second 24 hours and while no. 24 had a moderate diarrhoea lasting about 24 hours, the animal was not very sick and recovered rapidly. Its urine gave no indication of a diseased kidney. The amount of urine collected from nos. 24 and 25 was 360 cc. The leucin was isolated as above and 0.218 gm. obtained, $[\alpha]_D^{20}$ in 20 per cent hydrochloric acid = $-3.7^\circ (\pm 0.4^\circ)$.

The amount of urine obtained from rabbits nos. 17, 18 and 19 was 365 cc. This urine was treated in the same manner but after extraction with ether the residue was redissolved in water and treated with lead acetate before the esterification. The amount of leucin obtained was 0.161 g $[\alpha]_D^{20}$ in 20 per cent hydrochloric acid^o = $-6.8^\circ (\pm 0.4^\circ)$. It will be observed that the rotation of our products falls below that of the optically pure dextro leucin which is $[\alpha]_D^{20}$ in 20 per cent hydrochloric acid = -15.6° .³⁴

It was thought possible that part of the dextro leucin undergoes racemization in the process of isolation. Therefore mixtures were made of normal human urine and of water containing varying amounts of synthetic laevo leucin and the amino acid was isolated in the manner described. The results are given in the following table:

The laevo leucin used had a specific rotation $[\alpha]_D^{20}$ in 20 per cent hydrochloric acid = $-14.0^\circ (\pm 0.4^\circ)$

³⁴ Fischer und Warburg: Spaltung des Leucins in die optisch-activen Componenten, etc. Ber. der deutsch. chem. Gesellsch., 1905, 38, p. 3997.

LEVO LEUCIN ADDED IN GRAMS	URINE	LEVO LEUCIN RECOVERED IN GRAMS	LEVO LEUCIN RECOVERED IN PER CENT OF AMOUNT ADDED	$[\alpha]_D^{20}$ IN 20% HCL
0.100	200 cc.	0.041	41	Inactive
0.300	200 cc	0.076	25	+ 4.9° (\pm 0.4°)
0.600	200 cc	0.223	37	+ 6.9° (\pm 0.4°)
0.900	200 cc	0.443	49	+ 7.9° (\pm 0.4°)
0.100	water	0.077	77	+10.1° (\pm 0.4°)
0.201	water	0.167	83	+ 9.4° (\pm 0.4°)

This table shows that in the isolation of active leucin from the urine part of it undergoes racemization. The smaller the amount of active leucin present in a given amount of urine the greater the racemization. The presence of certain substances in the urine influences the yield of leucin. These results lead us to believe that in our experiments with rabbits no laevo leucin was excreted but only dextro leucin. The low rotation of the isolated product is due to a partial racemization in the process of isolation. The amount of the substance isolated was so small that it was useless to try to isolate the optically pure amino acid and to prepare the material for an elementary analysis. We feel justified however in stating that the substance isolated was leucin and also in concluding that the leucin was excreted in the dextro form. Wohlge-muth³⁵ recovered 2.5 dextro leucin from the urine of a rabbit which had received 10 gm. of the racemic compound per os. Abderhalden and Samuely³⁶ recovered 50-60 per cent of the introduced dextro leucin after feeding rabbits with 10 and 15 gm. of racemic leucin. Abderhalden und Kautzsch³⁷ state, a strong male rabbit did not excrete any dextro leucin when the quantity of the racemic compound given remained below 6 gm.

The following table contains the weights of our rabbits, the amount of racemic leucin absorbed, the amount of urine collected and the quantity

³⁵ Wohlge-muth: *loc. cit.* Ber. d. chem. Ges.

³⁶ Abderhalden und Samuely : *loc. cit.*

³⁷ Abderhalden und Kautzsch: *loc. cit.*

of dextro leucin isolated together with its specific rotation in 20 per cent hydrochloric acid.

RAB- BIT NO.	WEIGHT IN GRAMS	RACEMIC LEUCIN ABSORBED IN GRAMS	AMOUNT OF URINE	AMOUNT OF DEXTRO LEUCIN ISOLATED IN GRAMS	$[\alpha]_D^{20}$	REMARKS
13	1600	2.6	—	0.162	$-9.7^\circ (\pm 0.4^\circ)$	recovered
14	1700	1.37				
15	1450	2.53				
16	1420	3.73				
17	1240	2.92				
18	1400	2.97	365 cc	0.161	$-6.8^\circ (\pm 0.4^\circ)$	
19	1530	3.05				
24	1620	4.3				
25	1540	4.05	360 cc	0.218	$-3.7^\circ (\pm 0.4^\circ)$	Rec. 1.5 gm. benzoic acid per kg.

The doses of leucin therefore administered to the single animals were relatively small and normally, that is in absence of benzoic acid, a greater yield of leucin could hardly have been expected.

Several attempts to demonstrate the presence in the urine of benzoyl leucin were unsuccessful, while the presence of hippuric acid and of free benzoic acid could easily be shown by the method of Bunge and Schmiedeberg.³⁸

As stated above we feel justified in assuming that the leucin excreted by our rabbits was the dextro rotary modification. Consequently under the conditions of our experiments we have no indications that the organism of the rabbit poisoned by benzoic acid behaves differently to the optical isomers of leucin than in the case with the normal animal. It is possible that the examination of the urine of animals detoxified by the action of racemic leucin may yield a different result showing that under certain conditions the dextro leucin is utilized for the formation of glyco-coll instead of passing through the organism unchanged. The uncertainty of the detoxifying action of the racemic leucin prevented us from extending our investigations any further.

³⁸ Bunge und Schmiedeberg: Ueber die Bildung der Hippursäure. Archiv f. exp. Pathol. u. Pharmacol., 1877, 6, p. 233.

ON THE TOXICOLOGY OF THE TUTU PLANT

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One of the most interesting of the poisons to be obtained in pure condition within recent years is *tutin*, *tutu*, or the *toot poison*, the active principle of several species of *Coriariæ*, a group of plants found especially in New Zealand and causing the most serious pecuniary loss to the agriculturalist there, because of the many deaths which occur in stock from eating the various species. The *Coriariæ* are shrubs or small trees which grow to a height of three or four feet in certain varieties, or to a height of twenty to twenty-five feet in others. Both their succulent branches and their delicious berries are eaten with avidity by the domestic animals. The native Maoris have been familiar with the poisonous character of these plants since early times and from their language the term *toot* or *tutu* is derived. The early settlers in New Zealand quickly recognized the danger their stock was subjected to, and Lauder Lindsay (1) states that in 1863 the colonists uniformly lost a quarter of their animals from this cause alone, the percentage sometimes even reaching 75, and that the difficulty of keeping stock alive proved one of the greatest barriers to the settlement and development of the land. Lindsay further states that the animals brought originally by Captain Cook to New Zealand, sheep and goats, died in some mysterious way, and he identifies their symptoms as coming from "toot poisoning." The incoming Europeans quickly differentiated two varieties of poisonous *Coriariæ*, one the shrub proper *Coriaria thymifolia*, called by the Maoris *tutu-papa* or *tutu-heu-heu* (ground toot); the other the small tree or bush *Coriaria ruscifolia*, *tutu*, *pohou*, *tupakihi*,

(tree toot), and they further soon found that the tutu-berries were by no means an infrequent cause of death among human beings.

The various species of *Coriariæ* are identical in their poisonous action and no animal is naturally immune. Cattle and sheep especially suffer most severely, but larger animals are susceptible. An elephant belonging to a traveling menagerie, for instance, died of toot poison in seven hours, and the skeleton of this animal is now in the Colonial Museum in Wellington. Birds also are sensitive to the poison, and domestic fowls show symptoms of toot-poisoning from eating the berries. The number of cases on record of poisoning in man is not large, possibly twenty or twenty-five, but this is not surprising since the deadly character of the plant has been so well known for many years, and it presents no difficulties of recognition.

Besides *Coriaria ruscifolia* and *Coriaria thymifolia*, already mentioned, *Coriaria angustifolia* also is occasionally found in New Zealand and all three species are called tutu by the natives and by the settlers. Plants belonging to the same order are found in other countries having the same latitude, the best known of these being *Coriaria myrtifolia*, which is abundant in Europe. It is known in Germany as Gerberstrauch, or dyer's bush, and in France as redoul. It is largely used in the tanning of leather and as a black dye, and has considerable commercial value. The leaves are occasionally used to adulterate Alexandrian senna, and death has resulted therefrom. The active principle of this species is *Coriamyrtin*, a crystalline glucoside belonging to the pikrotoxin group of poisons and first isolated by the French chemist Riban (2). *Coriaria ruscifolia* is found in China, where it yields a black stain made use of by shoemakers, and the fruit of another species found in the Himalaya mountains is eaten. On this continent the species *thymifolia* is found in South America where it is known as the "ink plant," the juice of the fleshy petals being used as an ink under the name "chanchi" (3). The same species grows in Mexico, but no record of its poisonous action on animals has been found. I suspect, however, that some of the obscure cases of cattle poisoning described are due to this plant both in Mexico and in the

extreme southern part of the United States. The plants can be kept alive in this latitude only under artificial conditions in greenhouses, and while several representatives of the genus have from time to time been brought to the New York Botanical Garden and to Boston, none of these are alive at the present time. In London, however, in the Royal Botanical Gardens there are several specimens of *Coriaria* including both the *Coriaria myrtifolia* of Europe and the New Zealand varieties. In this latter country, although it is by all odds the most poisonous of all the plants found there, yet it serves also a useful purpose to the natives. The Maoris use the fruit as the source of a non-intoxicating beverage, musical instruments are made from the hollow stems, the juice is used for tattooing, and, because of its sweet taste, is mixed with drinking water and with jellies made from sea weed.

In animals poisoned by these plants the symptoms are largely referable to the nerve centers and consist of stimulated and then impaired respirations, tetanic convulsions and coma. The symptoms usually make their appearance within a short time after the plant is eaten, and lead to the death of the animal in a few hours. In man, recovery from severe poisoning occurs, but impairment of memory may result. Very many remedies have been tried without marked success. Bleeding is looked upon by stock-owners as the best method of affording relief and in human beings this has been combined with the use of stimulants, emetics, compulsory exercise, and chloroform to control the convulsions. The only drug which can be said to act as an antidote is *lime* or some of the other *alkalis*. *In vitro* tutu is destroyed by alkalis and the administration of lime has been carried out by Dr. James, of Wellington, in a case of poisoning from eating the berries (4). Lindsay states that carbonate of ammonia is a valuable remedy in animals suffering from toot poison. The earlier attempts to isolate the active principle of the toot plant by Skey (5), Hughes (6), and Cristie (7) were not successful, but in 1900 T. H. Easterfield, Professor of Chemistry in Victoria College, Wellington, and B. C. Aston, Chemist to the Department of Agriculture, obtained a crystalline glucoside from all three of the New Zealand species of *Coriariæ* (8). The fresh young shoots of the plants were finely

divided, the juice expressed, filtered, evaporated to a thick syrup, nearly neutralized by sodium carbonate and then shaken up with ether. A crystalline deposit occurred on evaporation of the ether and after further purification by recrystallization from alcohol, this material was found to be highly toxic for animals reproducing in them the symptoms found in animals accidentally poisoned by the plants. This substance has been named *tutu* by its discoverers. It is, according to these authors a colorless, odorless compound occurring in oblique-ended prisms. It is volatile, has an intense and lasting bitter taste and gives the chemical reactions of a glucoside. That is, it reduces Fehling's solution only after inversion by mineral acids, but then very strongly. It has the percentage composition of $C_{17}H_{20}O_4$. It is highly toxic, 0.129 gram killing a pig weighing 17 kilos in 5 hours, 0.01 gram killing a kitten weighing 1 kilo in 40 minutes and 1 milligram (.001 gram) causing a severe illness with convulsions in a cat weighing 2 kilos.

According to J. A. Gilruth, (9), Chief Government Veterinarian of New Zealand, the symptoms in the poisoned animals consist of tetanic convulsions, accelerated breathing, nausea, vomiting, spasm of the glottis and respiratory muscles, the animals succumbing in the height of the convulsions. The post-mortem examination reveals nothing beyond a few hæmorrhages in the submucous coat of the stomach and the stoppage of the heart in diastole. Prof. Marshall states that the pharmacological action of tutin is "closely allied to that of coriamyrtin, it producing salivation, a fall in the frequency of the pulse, increased respiratory activity followed by convulsions for the most part clonic and limited to the forepart of the body." Marshall thinks that the poison produces its effect by acting on the medulla oblongata and basal ganglia of the brain (10).

Recently through the kindness of B. C. Aston, Chief Chemist of the Department of Agriculture of New Zealand, I received a small quantity of crystalline tutin. The extreme toxicity of the substance for rabbits and guinea pigs and its localization in the brain and spinal cords of these animals, seem worthy of reporting.

TOXICITY

A small quantity of pure crystalline tutin, 0.15 grams, was dissolved in 30 cubic centimeters of distilled water to which a little ethyl alcohol had been added. The pure crystals dissolve with considerable difficulty in water, but the addition of the alcohol renders solution possible, although some time, 18 to 24 hours, is necessary before solution is complete. One cubic centimeter of this solution thus represents .005 grams or 5 milligrams of the pure poison. On the administration of this poison to both guinea pigs and rabbits, characteristic effects are almost at once apparent. With large doses the animals are thrown into violent convulsions within fifteen to thirty minutes. The respirations are labored, the head is retracted, and the limbs, especially the fore limbs, show peculiar clonic spasms. The animals run rapidly about their cages as though making the most violent attempts to escape, and even when the effect of the poison becomes more pronounced and the animals sink into a profound coma, the rhythmic movements of the fore legs are maintained almost to the moment of death.

The full effect of the poison comes on usually in about two hours, at which time the convulsions are most violent, and the animals succumb to the intoxication in from four to five hours. Death may occur much earlier from large doses, and rarely the animals sink into a profound coma, from which they never recover, without showing the preliminary stage of convulsions. With sublethal doses the animals show a peculiar somnolence, with occasional rapid movements of the fore legs, the symptoms passing off rapidly, and at the end of 24 hours the animals appear quite well, with no loss of weight, with natural movements and good appetite. There are no late effects and no evidence of a chronic intoxication such as develops when poisons are administered which produce degenerative changes of the parenchymatous organs. Guinea pigs and rabbits are equally susceptible to the poison, the convulsions, however, being somewhat more generalized in the larger animals, the guinea pigs always showing the peculiar running movement due to the clonic spasms of the fore limbs.

The toxicity of the poison for both rabbits and guinea pigs is very high, so high indeed as to make tutin rank as one of the most poisonous of organic substances, certainly one of the most toxic of the glucosides. The minimum fatal dose of guinea pigs of 250 gram, weight is about half a milligram, as can be seen from the following table:

TABLE I
Toxicity of Tutin for Guinea Pigs

DOSAGE	WEIGHT	EFFECT
1 cc. stock solution 5 milligrams	545 grams	Death 2 hours
0.5 cc. 2½ milligrams	290 grams	Death 2 hours
0.2 cc. 1 milligram	290 grams	Death 2 hours
0.1 cc. ½ milligram	275 grams	Death 2 hours
0.05 cc. ¼ milligram	315 grams	{ Somnolence and convulsive movements of fore-limbs. Complete recovery

With rabbits the minimum fatal dose is somewhat higher but bears a definite ratio to the body weight. For an animal of 1200 grams it may be considered about 2 milligrams.

TABLE II
Toxicity of Tutin for Rabbits

DOSAGE	WEIGHT	EFFECT
1 cc. stock solution 5 milligrams	1370 grams	Death 2 hours
0.5 cc. 2½ milligrams	1395 grams	Death 2 hours
0.2 cc. 1 milligram	1095 grams	Somnolence, no convulsions. Recovery.

RESISTANCE TO HEAT

Tutin is far more resistant to heat than the majority of organic poisons, and may be compared to some of the snake venoms in

this respect. It withstands boiling for half an hour, the boiled material showing apparently no loss in toxicity. Thus one cubic centimeter of our stock solution was boiled half an hour and, when administered to a guinea pig weighing 370 grams, killed it in violent convulsions in half an hour.

PERMANENCE OF ACTION

Tutin solutions preserve their toxicity for long periods of time without apparent deterioration. Our first tests were made in February, 1909. The same solution was again tested in November, nine months later, and found to exhibit the same or even a slightly increased toxicity, the solution being apparently slightly more concentrated from a little evaporation of the solvent. The slightly increased toxicity can be seen from the following table:

TABLE III
Toxicity of Tutin Solutions After Standing

DOSE	WEIGHT OF GUINEA PIG	EFFECT
1 cc.	500 grams	Death 2 hours
5 milligrams		
$\frac{1}{4}$ cc.	450 grams	Death 2 hours
$1\frac{1}{4}$ milligrams		
$\frac{1}{10}$ cc.	260 grams	Death 2 hours
$\frac{1}{2}$ milligram		
$\frac{1}{20}$ cc.	300 grams	Death 2 hours
$\frac{1}{4}$ milligram		
$\frac{1}{40}$ cc.	350 grams	No effect
$\frac{1}{8}$ milligram		
$\frac{1}{30}$ cc.	400 grams	No effect
$\frac{1}{15}$ milligram		

This same solution was tested in June, 1910, nearly a year and a half after its original preparation, and found to have its characteristic toxicity.

LOCATION OF TUTIN IN THE TISSUES

In animals dead from tutin intoxication, it is possible to locate the poison accurately in the nerve structures, to which it is closely bound. The poison may be identified by its chemical reactions. It fails to reduce Fehling's solution untreated, but after hydrolysis with mineral acids (preferably strong hydrochloric acid) gives a characteristic reduction with the deposition of the yellow oxide of copper. By this reaction it can be detected in the tissues and it is almost entirely limited to the brain and spinal cord. If these structures be emulsified with salt solution and boiled with hydrochloric acid, after neutralization, they give the reduction of Fehling's solution characteristic of the glucoside. Indeed these tissues seem to be loaded with the poison. Controls made with the brains and spinal cords of normal animals give but slight reduction of Fehling's solution, or none, even after prolonged boiling with hydrochloric acid. At times a little yellowish-green deposit is found in the bottom of the tubes, but when this is separated by filtration, decomposed by acetic acid and tested by ammonia for copper (cupra-ammonium oxide) the test is absolutely negative. Moreover, if the tissues of the brain and spinal cord are emulsified in salt solution and then filtered, the clear filtrate from normal animals gives no trace of reduction of Fehling's solution. Similar clear filtrates from both the brain and spinal cord of animals dead of tutin give the characteristic deposit of cuprous oxide when tested with the reagent. The blood of inoculated animals may show at times a faint trace of the poison, but the various organs, liver, kidney, spleen and adrenals, are quite free from it or at least show only those traces which can be accounted for by the amount of blood which they may contain.

The poison is clearly located then in the brain and spinal cord, and is closely bound to these tissues. If the brain and spinal cords of animals dead of tutin be removed aseptically and emulsified, this emulsion of tutin-loaded nerve tissue may be given to animals without the slightest effect. Not only is the poison localized in these structures, but it is bound to them in such a way as to be completely neutralized. Thus the brain of a 500 gram guinea

pig killed by the administration of five milligrams of tutin in half an hour, was emulsified in salt solution and the thick emulsion administered subcutaneously to a guinea pig weighing 400 grams. No effect whatever seemed to follow the inoculation. The same experiment was carried out in a number of animals, but in no case could any poisonous action be detected. Ocular inspection¹ of the amount of reduction of Fehling's solution produced by the nerve structures of the animals dead of tutin intoxication, as compared with the apparent amount of reduction produced by definite quantities of tutin would lead one to believe that the quantity of the glucoside present in the brain emulsions used in these experiments is much more than a fatal dose of the active poison and yet the animals showed no symptoms whatever, even the somnolence and the peculiar clonic spasms of the fore limbs produced by sublethal doses being absent. The poison seems to be so closely bound to the nerve structures or to be so altered in its chemical constitution either by the loss of certain constituents or by the assumption of some of the constituents of the tissues that it is no longer toxic. It may provisionally be spoken of as *detoxified tutin*.

Attempts to imitate in vitro this union of poison and nerve substance were unsuccessful. Aseptically removed brains and spinal cords fail to detoxify tutin and when emulsified with it and introduced subcutaneously into animals they die with all the signs of tutin intoxication. Various methods were employed. The tissues were emulsified with tutin solutions and injected at once, were preserved on ice for 18 hours and injected, were kept at 37° C. for the same length of time and injected, and in all instances the effect of the emulsion of nerve tissues and tutin was quite like that of the pure poison. Thus the brain of a normal guinea pig emulsified with one cubic centimeter of our stock tutin solution, and then kept on ice over night, was fatal to a 400 gram guinea pig in two hours, while another mixture of brain and one-half cubic centimeter of the solution, kept in the thermostat 18 hours killed a 370

¹ The quantity of tutin at my disposal was not so large as to admit of a series of quantitative estimations in respect to the point at issue.

gram pig in the same length of time. Not even a minimum fatal dose of the poison will be neutralized outside the body, since an emulsified brain mixed with two drops of tutin solution, representing the lowest limits of dosage of the poison for guinea pigs, and left in the thermostat over night, killed a 305 gram guinea pig in two hours.

The brains of animals removed from the body contain but relatively small amounts of blood serum and it was thought possible that some constituent of the serum might be necessary to effect a union of tutin and the nerve tissues, but no evidence could be adduced to show that blood serum has such an action "in vitro." Thus one and one-half cubic centimeters of fresh normal guinea pig serum mixed with two drops of tutin solution killed a 291 gram pig in two hours, and the same quantity of serum added to a brain emulsion and two drops of stock tutin solution, the mixture being kept on ice for 48 hours, killed a 420 gram guinea pig in two hours with the typical symptoms of tutin intoxication.

We are apparently unable to imitate outside the animal organism that peculiar process which in the body permits this powerful nerve poison to be localized and bound to the nerve structures and in such a combination to be completely detoxified.

IMMUNITY EXPERIMENTS

Gilruth (*loc. cit.*) has pointed out that animals recovering from non-fatal doses of the toot poison exhibit no subsequent resistance when inoculated with the fatal doses for normal animals. He reports the administration of one milligram of tutin to a cat weighing two kilograms and the production of convulsions and an illness lasting 24 hours. The animal recovered, but subsequently succumbed when inoculated with 3 milligrams of the poison.

The attempt was made to produce an immunity to this poison in both rabbits and guinea pigs, but with both species of animals the introduction of small non-fatal doses seemed to have no effect whatever in heightening the resistance of the animals to the action of fatal doses. After the effect of the poison wears off the animals are susceptible to apparently the same degree as are normal ani-

mals. There is no increased resistance, the animals dying of fatal doses for normal animals, and there is no evidence that the poison accumulates in the system. Even after the introduction of a number of small doses, over a fairly long period of time, it still requires a minimum fatal dose to bring on a fatal intoxication. The following tables indicate these points:

TABLE IV
Treatment of Rabbit

DATE	DOSAGE	WEIGHT	RESULT
March 30	1 milligram	1095 grams	Somnolence and recovery
May 4	1½ milligrams	1295 grams	Somnolence and recovery
May 31	2 milligrams	1450 grams	{ Typical symptoms of tutin intoxication and death in 2 hours

TABLE V
Treatment of Guinea Pig

DATE	DOSAGE	WEIGHT	RESULT
May 10	1 milligram	315 grams	No appreciable effect
May 31	2 milligrams		{ Death in 2 hours, with typical symptoms

Other animals treated with small doses of tutin at varying intervals likewise showed no evidence of increased resistance, all succumbing as soon as a normal fatal dose was administered. Even the administration of the detoxified tutin in the form of the emulsion of the brain of an animal killed with this poison, failed to induce any immunity. Thus on November 18th a 500 gram guinea pig was inoculated with one cubic centimeter of tutin solution and died in violent convulsions. The brain of this animal was removed, emulsified and injected into a guinea pig weighing 420 grams. No effect followed the injection and on December 10th one drop of the tutin solution representing $\frac{1}{4}$ milligram and on December 23rd two drops, representing $\frac{1}{2}$ milligram were administered, also without effect. On January 10th, 18 days after the last inoculation, the animal succumbed to 3 drops of the solution, representing about $1\frac{1}{2}$ milligram, a fatal dose for animals of its weight.

The failure to immunize animals to this glucoside is not without significance, and is in line with the work of Bashford, (11) and Ehrlich as to the character of the substances to which immunity can be produced. (12) It is Ehrlich's doctrine that the organic poisons of a firm fixed structure such as the crystalline glucosides form so intimate a union with the cells of the body that these cells fail to throw off any immune substances and the attempts of Bashford to immunize against a number of glucosides resulted in complete failure. It has been shown by the investigations upon the *Amanita-hæmolysin*, (13) however, that there are certain glucosides against which an artificial immunity can be produced, and the question arises, therefore, as to why it is that such a marked difference is shown by different representatives of this group. It may be pointed out that the *Amanita-hæmolysin* has many features in common with the true toxins. It is thermostabile, deteriorates rapidly on standing, is destroyed by acids and shows a definite latent period in its action upon animals. Tutin, however, is thermostabile, does not deteriorate on standing, acts upon animals without a latent period and forms an intimate union with the tissues of the body upon which its poisonous action is most pronounced. Tutin may be said to follow the general rule enunciated by Ehrlich as to the impossibility of immunizing against glucosides, while the *Amanita-hæmolysin* is an exception to this rule. Possibly the ease with which a poison may be broken up into its various components may be a more important determining factor in immunity production than the fact that it gives proteid rather than glucocidal reactions.

CONCLUSIONS

In conclusion, then, we have in *tutu* a crystalline glucoside whose toxicity will place it among the most poisonous of organic poisons, at least of poisonous glucosides. The poison does not deteriorate on standing and resists boiling. In the body it forms an intimate union with the nerve structures and in so combining is completely detoxified. This union and detoxification cannot be imitated outside the animal body. The administration of

doses causing symptoms of intoxication but not acutely fatal is not followed by secondary degenerative changes and the animals so treated recover completely. Finally such recovered animals show no increased susceptibility to the poison and exhibit no immunity against the action of normally fatal doses.

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- Abel and Ford: On the poisons of *Amanita Phalloides*. The Journal of Biological Chemistry, vol. ii, No. 4, January, 1907.
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ON THE ACTION OF MAGNESIUM SULPHATE

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Since the investigations of J. Loeb and his associates aroused a renewed interest in the action of certain of the inorganic salts upon protoplasm, placing the salts of magnesium, in particular, among those that exert a depressing influence upon living processes, the salts of magnesium have been made the subject of several pharmacological investigations, largely with the view to define their place as practical therapeutic agents.

At least as early as 1675 magnesium sulphate has been known to produce purgation when administered *per os*. In an effort to determine whether the purgative action would follow the intravenous or the subcutaneous injection of this salt, the older workers found that when so injected, magnesium sulphate was very toxic, producing no purgation, but producing a form of paralysis, accompanied with failure of respiration and cessation of the heart's action. Thus Jolyet and Cahours¹ showed that the intravenous injection of magnesium salts caused no purgation, but caused a loss of motility of the voluntary muscles. Matthew Hay² in his extensive article on the action of saline cathartics, showed distinctly that magnesium salts are very toxic when injected intravenously. He also observed that rapid injections of the salts are far more dangerous than slow injections. He caused the death of a 2.28 kilo cat by the rapid intravenous injection of 1.5 cc. of a 20 per cent solution of magnesium sulphate, while another cat survived 13 cc. of a 10 per cent solution injected slowly.

¹ Arch. de Physiol., vol. 2, p. 113, 1869.

² Jour. of Anat. and Physiol., vols. 16-17, pp. 243-405, 1882-3.

He noted that the muscles were relaxed and motionless and that respiration stopped while the heart was still beating strongly but slowly and that finally from sufficiently large doses the heart would stop in diastole without there being any convulsive or respiratory efforts whatsoever. He concluded that magnesium sulphate injected into the blood paralyzed first the respiration, then the heart, and that it abolished sensation, or at least paralyzed the sensory motor reflexes. His subcutaneous injections were in too small doses to cause much general effect. Leubuscher³ in a similar study found that magnesium sulphate injected intravenously was very toxic. Another of the important contributions of the earlier workers was that of Binet⁴ who claimed that magnesium salts caused paralysis of the nervous system. He says that magnesium agrees with lithium, potassium and sodium, in that it stops the heart in diastole; and that it is distinguishable from the other metals, barium, calcium, and strontium, by the quick paralysis of the peripheral nervous system, and that magnesium is a '*motor-paralyzant like curare*,' but it is distinguished from curare in that it allows breathing longer and at the end (in case of large doses) by paralyzing the heart and muscles like all metallic poisons.

These are sufficient to show that the older writers especially those of the French school, had a strong impression of the "curare-like" action of the salts of magnesium, while the toxic effects when injected intravenously, were well established. It might be mentioned, in passing, that none of these investigators attributed to them any true anæsthetic action.

More recently, Meltzer and Auer⁵ took the matter up and have made extensive studies on the action of magnesium sulphate and chloride. They confirm, very largely, the findings of most of the previous workers; and have added a few new observations and interpretations. They note the paralysis of the medullary centers, viz., respiration deglutition, and vaso-motor, as well as the general muscular paralysis which comes on before the medullary

³ Virchow's Archiv f. pathol. Anat., vol. 104, p. 434, 1886.

⁴ Rev. méd. de la Suisse romande, vol. 12, p. 523, 1892.

⁵ Amer. Jour. of Physiol., vol. 14, p. 366, 1906; vol. 15, p. 387, 1906.

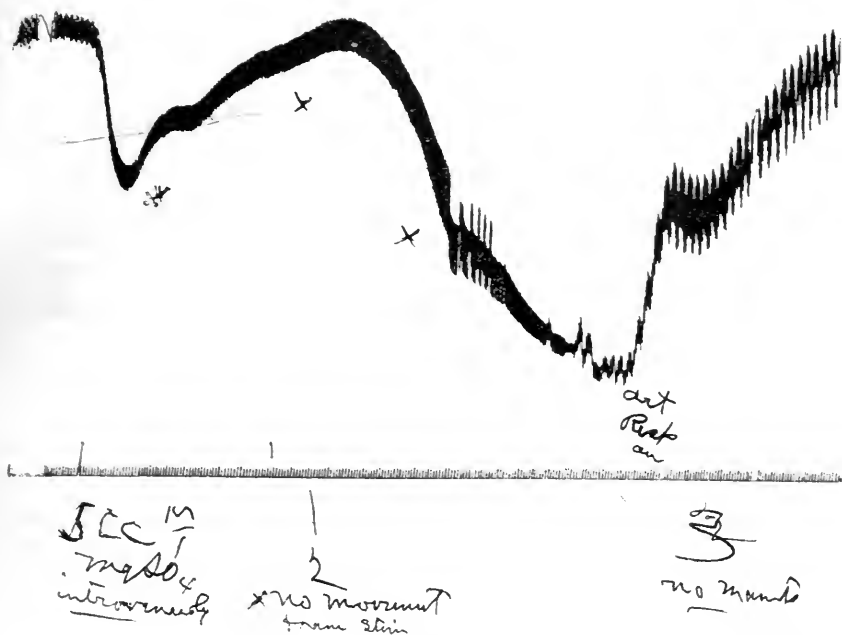


FIG. I. Blood-pressure curve after MgSO_4 . Dog wt. 5 Kilos. 5 cc. $\frac{1}{2}$. MgSO_4 injected into femoral vein. Read from left to right. At X, X, X, stimulated peripheral end of cut sciatic without response. Artificial respiration started at point so marked.

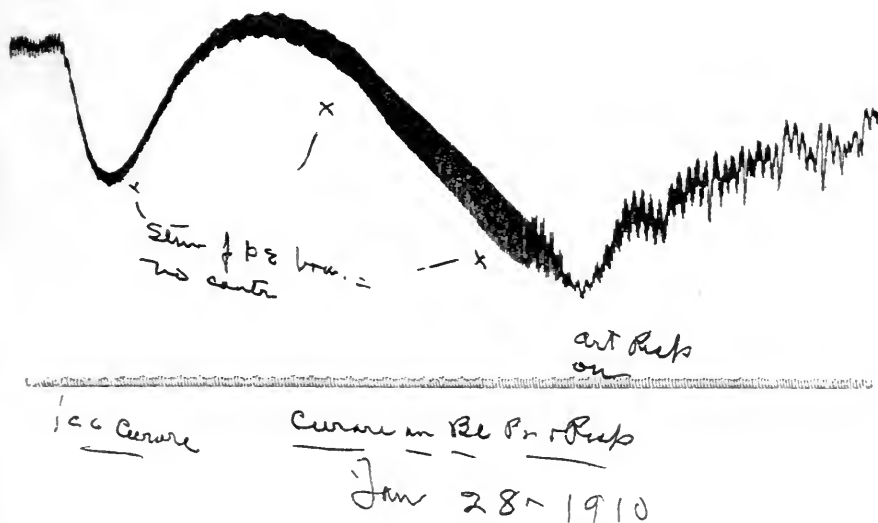


FIG. II. Blood-pressure curve after curare. Dog wt. 4.8 Kilos. 1 cc. saturated solution of curare injected into femoral vein. Read from left to right. At X, X, X, stimulated peripheral end of cut sciatic without response. Artificial respiration started at point so marked.

centers are depressed to a point incompatible with life. In this condition animals will endure surgical operations without external evidence of pain, and after a time will return to a normal state. This observation led Meltzer and Auer to ascribe a true anæsthetic action to these salts of magnesium. They also observed that when injected directly into the spinal canal (subarachnoidally) muscular relaxation and anæsthesia would follow.

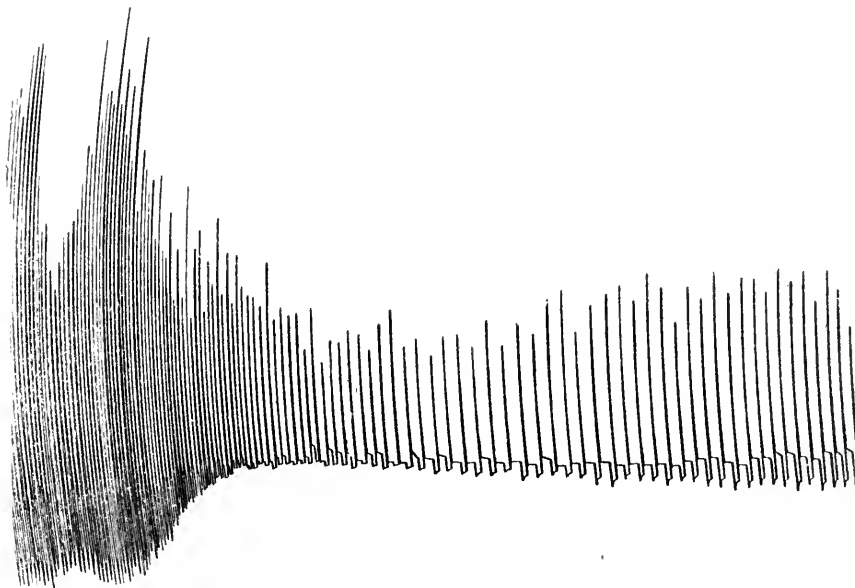


FIG. III. Respiratory tracing of dog showing effect of perfusion of head with MgSO_4 . 5 cc. MgSO_4 injected into carotid artery followed by marked slowing and decrease in amplitude of respiratory movements.

Wike,⁶ however, confirms the work of Binet and ascribed the phenomena observed to a curare-like action, or to a depression of the peripheral nerve mechanism.

Bardier⁷ also found that magnesium caused a depression of the peripheral nervous system and later the central nervous system.

⁶ Jour. de physiol. et de pathol. gén., vol. 8, p. 794, 1906.

⁷ Jour. de physiol. et de path. gén., vol. 9, p. 611, 1907.

QO A.

Very recently Guthrie and his pupils⁸ have confirmed the curare-like action, especially as set forth by Binet, Wike, and Bardier.

While the main object of our investigation was to determine, if possible, more accurately, the action of magnesium sulphate upon the heart, yet there seemed to be sufficient disagreement among investigators to justify us in making a series of experiments covering some of the other points under discussion.

The first point investigated was the cause of the sudden fall in blood pressure and the simultaneous cessation of respiration following immediately the intravenous injections of fatal doses of magnesium sulphate. From the fact that curare causes a similar drop in blood pressure with a simultaneous cessation of respiration, it was deemed advisable to compare the two drugs as to their mode of action on respiration and blood-pressure. When this was done the tracings showing the blood-pressure changes after fatal or almost fatal doses of magnesium sulphate and of curare were practically identical. In either case the respiration ceased in a few seconds and the blood pressure suddenly dropped and then began to rise. This rise, which is probably due to asphyxia, was followed by a second fall which gradually sank to the zero point and the heart would stop altogether unless artificial respiration was resorted to. If artificial respiration was employed, the heart would begin to beat strongly, and the blood pressure would return to normal. (Figs. I and II.)

The fall in blood-pressure and cessation of respiration in curare poisoning is known to be due to a peripheral paralysis of the motor nerves. Is the same true of magnesium sulphate? To test this we made the following experiments: The right femoral vein of a dog was transected and a T-cannula interposed between the cut ends, restoring its continuity, so that the circulation could go on as usual or else by placing a bull-dog clamp on the vein on the proximal side of the T-cannula and opening the sidearm of the cannula, the blood returning from the limb could be drained off. By injecting it into the femoral artery, the magnesium sulphate would circulate through that limb only, and be drawn

⁸ Proc. Soc. Exp. Biol. and Med., vol. 7, p. 39, 1910.

out at the T-cannula. Immediately after such perfusion the irritability of the sciatic nerve was tested by stimulating with interrupted induction shocks, and the strength of stimulus necessary to produce clenching of the toes compared with that necessary to cause a similar reaction before administration of the magnesium sulphate. It was found that the slow (2 minutes) perfusion of 5 cc. $\frac{N}{1}$ $MgSO_4$ solution (it being immediately drawn out at the femoral vein so that it simply circulated through once) was followed by a marked depression of the peripheral motor nervous mechanisms when tested as described above. The depression was almost immediate (as soon as we were able to make the test) and lasted for about fifteen minutes at which time recovery began to occur and was complete in about thirty minutes. Experiments of this kind showed that there was a marked depression (curare-like action) on the motor nerve endings.⁹

The next point for attention was whether the peripheral depression and subsequent recovery occurred concomitantly with the stoppage and return of respiration. On observation of both peripheral motor responses to electrical stimulation and respiratory movements, it was found that they practically accompanied one another. This is shown by the following experiment:

An 8 kilo dog was prepared for respiratory tracings and for stimulation of the sciatic nerve; the femoral vein was exposed for the injection of magnesium sulphate, and the stimulus just sufficient to cause "the toe clench" was determined. 5 cc. of an $\frac{N}{1}$ $MgSO_4$ solution were injected into the femoral vein in two minutes. Generally this dose was sufficient to stop respiration and cause a marked fall in blood pressure by the time the injection had been completed. Immediately upon stimulation of the distal end of the cut sciatic with the same strength of current that previously gave a good contraction, now caused no contraction at all.

⁹ This is not in keeping with the statement made by one of us (Matthews, Amer. Jour. of Physiol., vol. 21, p. 5, 1907), that the muscular response to stimulation of the sciatic nerve was not depressed. The error was due to the fact that the observations were made too long after the time of the administration of the magnesium sulphate, thus allowing the nerve time to recover, or almost recover, before making the test.

Upon increasing the strength of the current, by moving the secondary coil seven centimeters nearer to the primary, a weak response was elicited, thus showing that the motor response was not entirely abolished, but greatly depressed. In about fifteen minutes (artificial respiration being used in the meantime) return of the irritability of the sciatic nerve began to be noticeable. Just about at this point, when artificial respiration was stopped, very weak, slow, jerky, respiratory movements appeared. After thirty minutes from the time of the injection of magnesium sulphate, both the motor responses and respiration had returned to normal. In these experiments the disappearance of respiration accompanied the depression of motor response and return of respiration accompanied the return of motor response.

These results show conclusively that the peripheral motor nerve depression is amply sufficient to account for cessation of respiration in magnesium poisoning, just as in curare poisoning. But this does not prove that there is no central action; it only shows that it is not necessarily a central action.

In order to get at this point more directly, the head and neck of one dog was perfused with blood from the carotid of another dog into which sufficient magnesium sulphate had been injected to stop respiration. In this way the magnesium sulphate blood would circulate through the perfused head, but not through the body belonging to that head. This would leave the peripheral neuro-muscular apparatus unaffected, and any depression occurring would be due to direct action upon the respiratory centres.

Two dogs were anæsthetized and placed side by side. In one (dog A) all the vessels traversing the neck were tied off, except one internal jugular and one common carotid. That is, the vertebrals, both external jugular veins and one common carotid were tied off. The other internal jugular and carotid were prepared for the transfusion. This would shut off all the circulation between the head and the body except what would go through the spinal canal, which is a very considerable amount in most dogs; but it would certainly reduce the exchange of blood between these parts sufficiently to distinguish the head effects from the body effects, or central effects from peripheral effects.

Dog *B* was then connected by cannula to dog *A*, so that the blood from one of its carotids entered the head of dog *A* through the one of its carotids as previously mentioned, and would return through the internal jugular of dog *A* which was connected to the external jugular of dog *B*. After the cross circulation had time to become established, sufficient $\frac{M}{1}$ solution of magnesium sulphate was slowly injected into the femoral vein of dog *B* (the donor) to stop its respiration. This had no noticeable effect on the respiration of dog *A* (the recipient). By keeping up artificial respiration in *B*, the heart continued to beat strongly. More magnesium sulphate was injected until the heart began to fail; but this produced no noticeable effect upon the respiratory movements of *A*. These experiments indicate that the dilution in which the magnesium sulphate finally reached the brain of dog *A* was so great that there resulted no marked action upon the respiratory centre. It is possible that the magnesium became fixed in the tissues of dog *B*. As a control of this point, the magnesium sulphate was injected directly into the short rubber tube connecting the carotid arteries of the two dogs, so that it would pass first through the perfused head (dog *A*) and then back into the external jugular vein of the donor (dog *B*). When this was done, 5 cc. of an $\frac{M}{1}$ $MgSO_4$ solution was usually sufficient to cause a marked depression of the respiratory movements (Fig. III), the tracing showing both slowing and decrease in the amplitude of movements. 10 cc. $\frac{M}{1}$ $MgSO_4$ so injected was sufficient to stop the respirations entirely in dog *A* and also in this case to paralyze simultaneously both the heart and respiration in dog *B*. This was probably due to the fact that the magnesium salt, after traversing the head of dog *A* returned through the external jugular vein of dog *B* and entered the heart in a relatively concentrated solution. However, it sometimes occurred that the heart and respiration stopped simultaneously and even very rarely the heart ceased first.¹⁰

These results indicate that the respiratory centre is susceptible to the depressing action of magnesium sulphate, but that it does

¹⁰ Mickwitz, Dissertation, Dorpat, 1874.

not respond so readily as do the motor nerve endings. A concentration of magnesium sulphate sufficient to depress the motor nerve endings to a degree incompatible with respiratory movements will not stop the respiration when the peripheral nerves are protected against the action of the magnesium salt. This is more or less in accord with the findings of Cristau.¹¹ But in some of the experiments where the magnesium sulphate was injected directly into the ear vein of an unanæsthetized dog, somewhat different results were obtained, indicating that there may be even a primary stimulation rather than the depression of the respiratory centre. When injections of about 10 cc. of $\frac{M}{2}$ $MgSO_4$ were made in one minute into the ear vein of a dog, weighing about 10 kilos, the dog showed increased respiratory movements and nausea and vomiting. Of course, in such experiments, the animal being fully conscious, the effect of the handling and injection of the dose, and voluntary movements, may have been sufficient to account for the effects at first observed, though injection of other salts, calcium chloride, sodium sulphate, sodium citrate and sodium chloride made with the same technique, showed no such action upon the respiration.

These experiments indicate that the most marked action of magnesium sulphate is a curare-like action, that is to say, a paralysis of the motor endings. The effect upon blood pressure is identical with that of curare. In sufficient doses, there is also depression of the respiratory centre; but not when given in doses sufficient to greatly depress the peripheral neuro-muscular apparatus. On the other hand, when given intravenously to the unanæsthetized dog, there is evidence that it may cause primary stimulation of the respiration.

Since magnesium is shown to depress the peripheral motor nerve endings, and in this way produce motor paralysis, there remained yet to be investigated its action upon the sensory endings. On this point we have not as yet obtained conclusive results on dogs, but have some evidence that the sensory endings also are slightly depressed by the perfusion of magnesium sulphate.

¹¹ Doctor's thesis. U. of Lyons, 1907.

We attempted to perfuse one hind leg of the dog with magnesium sulphate, as above described, by injecting the salt solution with a hypodermic syringe into the femoral artery of the leg and drawing off the blood from the femoral vein in order that the salt might act only upon this leg and not enter into the general circulation, and then by stimulating the perfused foot with adequate stimuli that would give a good crossed reflex before the perfusion with magnesium to note whether there was any change in the response after perfusion, but the dogs so tested did not give very conclusive results.

In a series of five dogs, weighing about 10 kilos each, which were used without an anæsthetic, three successive doses of 10 cc. $\frac{1}{2}$ MgSO_4 were injected into the ear vein at 15 min. intervals. These injections caused, in addition to the effects mentioned above, muscular weakness and depression of all muscular movements going on to paralysis, but with good preservation of the corneal reflex, even at a time when cutting of the skin could be done without bringing out resistance from the animal.

On frogs the results indicate quite clearly that in sufficient doses magnesium sulphate paralyzes the motor endings without distinctly depressing the sensory mechanism. Repeating Claude Bernard's curare experiments, but using magnesium sulphate instead of curare, showed that good cross reflexes could be obtained by stimulating the unligated (curarized) leg which caused contractions in the ligated (normal) leg. The stimuli used were electrical and thermal and mechanical.

These results indicate that magnesium sulphate does not markedly depress the sensory apparatus when administered in amounts sufficient to paralyze the motor endings.

From the above, it would seem to be possible by the administration of MgSO_4 to separate physiologically, the skeletal muscles from all of their nerve connections, or in terms of depression to lessen the muscular response to indirect stimulation to almost any degree desired.

In regard to the heart, have we any grounds to ascribe the action of magnesium salts as being due to a nerve depression? Is it possible to separate the heart muscle physiologically from

its intrinsic nervous mechanism; and if so, will it retain its automaticity?

The heart failure as described by the older workers and more in detail by one of us¹² is ushered in first by slowing and moderate dilation, soon followed by incoordinating beats, the incoördination generally partaking at first of the form of two auricular contractions to one ventricular contraction and as it progresses, the heart may assume a rhythm of 2 to 1, 3 to 1, 4 to 1, etc. Sometimes the auricles continue to beat normally while the ventricles remain quiescent. At other times the auricles and ventricles continue to beat with independent rhythm, the auricles more nearly retaining the normal rhythm. The final result is a sudden dilatation and complete standstill. One of the characteristics of the heart after it has become quiescent, soft, and dilated is, the muscle is susceptible to stimuli and may quite readily be thrown into delirium cordis. If the heart be kept beating for ten to fifteen minutes by single induction shocks (100 times per minute) or by tapping, it will resume spontaneous beats, which are slow and incoördinated at first, but which soon return to normal. The return of the heart to its normal rhythm corresponds rather closely in point of time and character to the return of the peripheral motor response.

The behavior of the heart suggests, first, a depression in tone (dilatation, weakness, and slowing); second, some interference with the conductions of the cardiac impulse (incoördination, independent rhythms in auricles and ventricles and partial and complete heart-block); and third, a depression of all spontaneous movements.

In the final state in which the heart is also lutely quiescent, stimulation of the accelerator nerves of the heart will arouse it to regular efficient beats (fig. IV, *a, a, a, a, a,*). In these experiments the right accelerator was stimulated usually just below the inferior cervical ganglion. After the heart was in complete standstill, mild stimulation of the accelerators was begun. This always caused beats of something near the normal rhythm and strength.

¹² Matthews, S. A., Amer. Jour. of Physiol., vol. 20, p. 323, 1907.

The stimulation was continued, with occasional interruptions long enough to ascertain whether the heart would beat automatically or not, for ten to fifteen minutes, after which time when the stimulation of the accelerators was suspended, the heart would begin to beat automatically, with first a few powerful beats with long pauses between, after which it would start off on its normal auricular-ventricular rhythm (fig. IV).

This is an instance where the quiescent heart can be started up solely by stimulation of the accelerator nerves, contrary to the statement of Gaskell.¹³

Certain drugs are of great assistance in restoring this automaticity of the heart after magnesium standstill. One of the most efficient of these is adrenalin. If one drop of $\frac{1}{1000}$ solution of adrenalin in a few cc. of 0.9 per cent sodium chloride solution be injected with hypodermic syringe directly into the right ventricle of the quiescent heart and light massage performed sufficient to cause one or two preliminary beats, then the heart will immediately start off on a regular, powerful series of beats; or if instead of massage the accelerators be stimulated but for a short time, the heart will start off on automatic rhythm. Barium or calcium have a similar influence, but are not so efficient. Atropin added to any of these will greatly increase its efficiency.

What is the explanation of these reactions? Since it is shown that magnesium depresses especially the motor nerve endings, and also probably slightly the sensory nerve endings, or in other words, is a somewhat general nerve depressor, it may be assumed that the nerve endings in the heart are depressed to a point below which they can respond to the normal physiological stimuli. This would leave the heart bereft of all nervous influences, and in the state comparable to that of the limulus heart with the dorsal nerve cord removed. Not that the nerve elements of the heart are permanently removed or destroyed, but that they are temporarily depressed to a point where they are below the threshold of the normal stimuli. This is indicated by the fact that the heart can be made to beat by stimuli that are greater than those normally passing over the nervous mechanism of the heart, and also by the

¹³ Schäfer's Text-book of Physiology, p. 218, 1900.

further fact that those drugs that are known to heighten the irritability of nerve receptive substances are efficient in aiding in the restoration of the normal beat of the heart, presumably, especially in the case of adrenalin, by bringing the irritability of the neuro-muscular elements back to a point where they are again able to transmit their normal impulses, and presumably, in the case of barium by heightening the irritability of the heart muscle which in this way produces practically the same result. Atropine's efficiency depends upon the fact that it removes all remaining inhibitory influences leaving the heart in a condition where it is rendered more susceptible to impulses from the accelerators. As magnesium depresses the motor nerve endings of skeletal muscles, it seems that it also depresses the motor nerve endings of the heart, and that when these nerve endings are depressed below a certain point, the heart muscle has no more power to contract spontaneously than has a skeletal muscle. From these considerations it appears that the most satisfactory and harmonious explanation of the mechanism of the action of magnesium upon the heart is based upon the neurogenic theory of the heart's automaticity.

These experiments suggest that the action of magnesium sulphate upon the heart is essentially a depression of the cardiac nervous mechanism, especially the accelerators, resulting in a loss of tone, incoördination, leading to loss of automaticity; but at the same time retaining its irritability.

SUMMARY

1. These experiments tend to confirm the curare-like action of magnesium sulphate, particularly as described by the French authors.

2. Magnesium sulphate in doses sufficient to give marked action upon the motor endings, produces little effect upon the respiratory centre; but in larger doses, it causes depression of the centre. When administered to the unanæsthetized dog, primary stimulation of the respiratory movements were observed.

3. The action of magnesium upon the heart is best explained as that of a depression of the cardiac nervous mechanism involving particularly the accelerators.

ON THE EFFICACY OF ANTIMONY-THIOGLYCOLLIC ACID COMPOUNDS IN THE TREATMENT OF EXPERIMENTAL TRYPANOSOMIASIS

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Numerous efforts are being made to improve the treatment of the diseases caused by the flagellate protozoa known as *trypanosomes*, diseases which are among the most fatal and widespread of those known to medicine.

Among the best understood of the trypanosomic diseases are the sleeping sickness of human beings which is rapidly extending its ravages in Africa, the tsetse-fly disease, or nagana, which occurs in the horse, mule, donkey, in cattle and in other animals, *surra*, an Asiatic disease of the equidae, of camels, and of cattle, the epizootics of equines known as *mal de caderas*, *dourine* and the Gambian horse disease, and also the epizootic of the bovidae known as *galzielte*, or gall-sickness.

A number of investigators have been fairly successful in the treatment of mice and rats which had been infected with the trypanosomes of these diseases, but the attempts to cure man and the larger animals, as horses, cattle, or even dogs, have not met with equal success. With the drugs at present at our disposal, the outlook for a permanent cure¹ in man is not hopeful if there are signs of a wide-spread invasion of parasites, particularly if the cerebro-spinal system is so far involved that the spinal fluid,

¹ The difficulty of curing animals and men in the later stages is perhaps largely due to the fact that we here encounter stages of development in the reproductive cycle of the parasites which for one reason or another are probably not easily destroyed by the chemo-therapeutic agents at our disposal. See, for example, Moore, Nierenstein and Todd: *Ann. of Trop. Med. and Parasit.*, I, p. 283; and Dutton, Todd and Hanington; *ibid.*, I, p. 20.

as obtained by lumbar puncture, gives histological evidence that the meninges have been invaded even though the parasites themselves may not be present in the specimen of fluid which is being examined.

Not only have the drugs now in use proved to be inefficient in sterilizing all of the tissues when once this widespread invasion obtains, but they have all developed untoward effects of so serious a nature that it has become necessary to search for new drugs which shall combine greater efficiency as parasitocides with fewer dangers to the host, or which shall possess even such minor elements of superiority as adaptability for subcutaneous administration, or some other advantage which shall make them suitable for use as substitutes for those now in use. Our own work constitutes a contribution in this direction. The antimonials that we have employed in our experimental work are well adapted for subcutaneous or intravenous medication. It remains to be seen whether they have any advantages over the drugs now in use in the treatment of human beings, but we believe that our results are such as to warrant a trial of these drugs in the case of human trypanosomiasis as well as in the trypanosomic diseases of the domestic animals.

It is not our purpose to give a detailed history of therapeutic knowledge in regard to trypanosomic diseases. The publications of the Sleeping Sickness Bureau, the reports of Sleeping Sickness Commissions, the various special journals devoted to tropical diseases, and the various treatises on tropical medicine give full accounts of the brilliant work that has been done in this field since David Livingstone (1) in 1858 first suggested the use of arsenic in the tsetse-fly disease and tried it on a mare.

Various compounds of arsenic and of antimony and a number of dyestuffs have been brought forward as the result of the labor of laboratory workers and of physicians who have tested these substances in cases of sleeping sickness. As we are here more immediately concerned with compounds of antimony, we shall not enumerate the various arsenicals, dyestuffs and other drugs that have been or are being employed as trypanocidal agents, but shall refer the reader to the sources of knowledge already enumerated (2).

The introduction² of certain salts of antimony into this branch of therapeutics by Plimmer and Thomson, acting upon a suggestion made to them by Cushny, was a distinct advance. These observers (3) made use of the antimonyl tartrates of potassium and sodium in the treatment of rats infected with nagana or surra, and were impressed with the quickness with which these drugs caused the disappearance of trypanosomes from the peripheral blood even when it was "swarming with the parasites." A dose of 0.35 cc. of a 1 per cent solution caused their entire disappearance from the blood within half an hour. Recurrences, however, were observed in 4 out of 25 infected rats. Mesnil and Brimont (4), who also and independently of Plimmer and Thomson made use of tartar emetic, obtained better results with this drug in the treatment of rats infected with *T. evansi* than with those infected with *T. brucei*, and also noted that this antimonial was trypanocidal for an atoxyl resistant strain of surra.

Uhlenhuth and Woithe (5) were less successful than Plimmer and Thomson with sodium antimonyl tartrate in the case of rats infected with *T. equiperdum*, but these authors, who were more especially concerned with atoxyl, speak of their experiments with the tartrate as "experiments of orientation" and advise the further study of antimonials especially in connection with atoxyl.

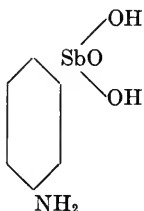
Breinl (6) was less successful with sodium antimonyl tartrate in the treatment of rats infected with a fairly virulent strain of *T. equiperdum* and stated that the local effects of the drug in rats were very severe, causing necrosis and sloughing, but nevertheless this investigator considered "that the introduction of another metal

² It would be more correct to speak of the *reintroduction* of this drug into the therapeutics of infectious diseases. It is of course well known that the *omne in omnibus* of Basil Valentine has had a wide use in the past in the treatment of agues, lobar pneumonia and other febrile diseases. In an essay published anonymously in London in 1747, (see Index Catalogue of the Surgeon Generals' Library) by an author who styles himself "An Eminent Physician," "the celebrated drug" is vaunted on the title page as "one that is not only an effectual cure for the present distemper among the cattle but a preservative from their being infected." And in the body of the work it is stated that, "It (antimony) is esteemed and daily used by the farriers as a kind of panacea for horses. It cures those obstructions of the lungs that make them shortwinded, heals the scab and cutaneous diseases, and helps very much to bring an emaciated jade to good plight again."

(antimony) belonging to the same group as arsenic is a further progressive step in the treatment of sleeping sickness and is moreover very suggestive."

From this time on progress in the study of the action of antimonials has been continuous and many advances have been made. Plimmer (7) and his associates have continued their studies on the behavior of various antimonials in rats and dogs and have also made important experiments "in order to find out where the trypanosomes rest during the period in which the peripheral blood is free from them after treatment with antimony." In conjunction with Bateman, Plimmer finds in rats that have been treated with antimony that trypanosomes live longest in the bone marrow and that the liver is also a place where these organisms find protection.

Breinl and Nierenstein (8) have recently extended our list of organic antimonials by preparing antimony compounds which are analogous to atoxyl. They have succeeded in preparing *p*, *m*, and *o*-amino-phenylstibinic acids. The action of these compounds was studied on animals infected with various strains of pathogenic trypanosomes. The *o*-compound was discarded after a few trials as impracticable. The *m*-compound caused marked swellings and abscesses and induced severe hæmorrhagic nephritis and seems to have given only discouraging results. The authors point out that the corresponding *m*-amino-phenyl arsenic acid was found by Ehrlich also to be markedly inferior in therapeutic value to *p*-amino-phenyl arsenic acid (atoxyl). *P*-amino-phenyl-stibinic acid



was administered in the form of its sodium salt to rats, dogs and monkeys infected with *T. brucei*, *T. evansi* or *T. gambiense*, and the results showed that this compound is a fairly powerful trypanocide, though its action is not as rapid as that of sodium anti-

mony] tartrate. A trial of this compound is considered to be justifiable in patients suffering from sleeping sickness. It is also pointed out that a careful systematic examination of the urine is advisable inasmuch as kidney lesions are among the most pronounced results of antimony poisoning.

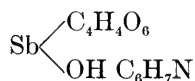
In this connection it may be noted that Kopke (9) has recently given a preliminary account of the action of this remedy in a case of sleeping sickness in a human being in which atoxyl had absolutely no effect on the parasites, and found that it caused them to disappear promptly from the peripheral circulation. The hypodermic administration of the remedy induced only a small amount of local swelling but caused severe pain persisting for several days.

Thomson and Cushny(10) have tested a number of antimony compounds on nagana rats. Among those that were inefficient, impracticable, or valueless for one reason or another may be named diphenylstibinchloride ($(C_6H_5)_2SbCl_3 \cdot H_2O$), potassium metantimoniate (SbO_2OK), a colloidal preparation of antimony oxide (Sb_2O_3) and a glyceride of antimony analogous to boroglyceride. Schlippe's salt, sodium sulphantimonate ($(NaS)_3SbS$), destroyed the trypanosomes in the rat, but induced very considerable local reaction, the results here being in agreement with those obtained with this salt in man by Brodenand Rodhain (11). Better results were obtained with sodium antimonyl malate and with solutions of ethylantimonyl tartrate prepared by Collie by heating freshly precipitated antimony oxide with ethyl tartrate to about $150^\circ C$. in sealed tubes. While tartrates and malates in the form of their sodium and potassium salt were found to be equally efficient as trypanocides, it was thought that the substitution of an alkyl radicle, as in ethylantimonyl tartrate, was attended with some advantage. Solutions of this compound, either as such or neutralized with ammonia, induced no local reaction in rats and caused the trypanosomes to disappear from the peripheral blood within one or two hours. Thomson and Cushny conclude that in their experiments no other drug has given such favorable results. Out of 13 rats treated with a single full dose of this drug, 6 showed a recurrence on the 14th, 16th, 22d, 26th and 29th days. There was no recurrence in the 7 others. Of these one died of pneu-

monia on the 84th day and 5 others from exposure to cold between the 135th and 165th day leaving 1 survivor after 260 days.

The strain of nagana here used was fatal to rats within 6 days after inoculation. As we shall see later a single full dose of a given drug is the more likely to be followed by a recurrence of the parasites in the blood the shorter the time that elapses between the injection and the usual time of death (in infected but untreated animals).

Laveran (11^a) also has lately introduced a new antimonial into this field, Aniline antimonyl tartrate, which was first prepared by C. S. Evans who ascribes to it the formula



Laveran obtained such good results with this compound in the treatment of guinea pigs infected with *T. evansi*, *T. gambiense*, *T. peccaudi* or *T. soudanense*, that he was induced to send some of the drug for trial in the field to Dr. A. Thiroux who has charge of a segregation village for sleeping sickness patients in Senegal near St. Thomas. The compound is less toxic than tartar emetic, and Thiroux finds that an intravenous injection of 0.150 gm. suffices to drive the trypanosomes from the peripheral circulation. It also seems to have a more decided action on the central nervous system than atoxyl, for two individuals who were just entering upon the last or drowsy stage of the disease are reported to have greatly improved after an injection of 0.150 gm. It remains to be seen, as Laveran says, whether these satisfactory preliminary results will be found to be lasting.

It will be of interest to note what results have been obtained in the treatment of human trypanosomiasis by the antimonials which were first introduced. Manson (12) was the first to administer sodium antimonyl tartrate to a case of sleeping sickness and his experience led him to say "that antimony may have a therapeutic influence in trypanosomiasis, but the hypodermic injection of the sodium tartrate of antimony is impracticable." The injection causes intense irritation and pain.

Broden and Rodhain (13) were the first to use tartar emetic with

encouraging results. These authors administered the drug intravenously in doses of 0.1 gm. A few of the patients thus treated responded with marked sweating and vomited two or three times. These symptoms disappeared after a few minutes. When this quantity of the drug (0.1 gm.) was given once a day for 8 to 10 days in succession, a large percentage of the patients showed signs of poisoning—they lost weight, had no appetite, and frequently complained of feeling ill. More than 10 successive injections could not be tolerated. After the drug was discontinued the untoward effects that have been named rapidly disappeared.

G. Martin and Leboeuf and L. Martin and H. Darré have also administered tartar emetic (0.1 in 100 cc.) in this way in numerous cases of sleeping sickness and have added to our knowledge in regard to its therapeutic limitations, and Thiroux has written a paper dealing with the untoward effects that are seen when tartar emetic is administered intravenously. It is pointed out by Thiroux (14) that tartar emetic should only be injected in very dilute solutions. Injections of 0.1 gm. in 200 cc. of fluid may be followed by a filiform pulse, vertigo, profuse perspiration, spasmodic cough, syncope or only momentary unconsciousness. These symptoms all pass off in a short time but the patient may feel more or less ill for a day. Thiroux seems to think that tartar emetic may have caused the death of one of his patients who had no lesion of the heart but nevertheless died from heart failure five days after having received intravenously a single dose (0.1 gm.) of tartar emetic. This author now gives caffeine (0.02 gm.) subcutaneously 20 minutes before injecting the tartar emetic, and states that his patients no longer evince untoward symptoms, especially if care be taken to inject very slowly.

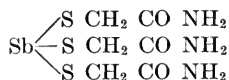
It will thus be seen that the untoward symptoms that follow *immediately* upon the too rapid intravenous injection of tartar emetic may be avoided by the proper attention to details. Not so, however, with the damage that tartar emetic may inflict upon the kidneys, the liver or the gastric tract *when the drug is given in full doses for too long a period*. These more serious results can only be avoided by reducing the dose or by substituting from time to time for tartar emetic other antimonials or drugs that are less toxic.

Martin, Leboeuf and Ringenbach (15) have tested the combined atoxyl-emetic treatment in advanced cases of sleeping sickness. Thirty-one patients of whom only one was in the first stage of the disease, were treated, with the result that 10 died within a short time after treatment was begun. The conclusion of these investigators is that the atoxyl-emetic combination does not succeed in staying the advance of the disease in patients who have reached the second stage and as such patients constitute the majority of cases seen among the natives, the great desideratum is a rapid and easily applied cure for these cases.

It will thus be seen that the antimonials at present in use are limited in number, and like the arsenicals and other drugs that have been put to a searching practical test in human trypanosomiasis, they are of doubtful efficacy except in those cases which are still in the earliest stage of infection. Nevertheless the search for specifics against trypanosomes must go on, and even if the newer drugs have only a slightly less toxicity or some such advantage as greater penetrability for the central nervous system or better adaptability for subcutaneous administration, they will deserve to be tested in the trypanosomiasis of human beings or of the larger domestic animals under the conditions that prevail in the infected regions of tropical countries.

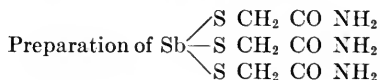
THIOGLYCOLLIC COMPOUNDS OF ANTIMONY

During the past year one of us (A) has prepared the triamide of antimony thioglycollic acid.



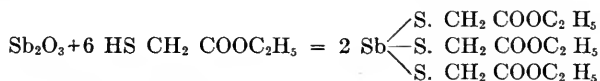
This compound is obtained in the form of a thick syrup or semi-resinous body which is soluble in water in all proportions, is powerfully trypanocidal, and may be administered subcutaneously in solutions containing 0.010 gm. to the cubic centimeter without signs of pain or local irritation. The compound tends to deposit a little sulphide of antimony on long standing and this is readily removed by filtration. *Only solutions that have been made perfectly*

clear by filtration should be injected subcutaneously or intravenously. As in the case of antimonials in general only extremely dilute solutions, that is only such as contain one-half or at most one milligram in the cubic centimeter, should be injected intravenously if one wishes to avoid untoward effects like those that were cited in speaking of the clinical use of tartar emetic.



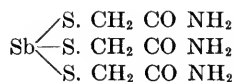
The first step in the preparation of this compound is the preparation of pure thioglycollic acid. For this we followed the directions given by Klason and Carlson (16) and prepared the crystalline barium salt ($\text{Ba S CH}_2\text{COO} + 3 \text{H}_2\text{O}$) and from this obtained the free acid which was then purified by distillation under a barometric pressure of 14-16 mm. and at a temperature of 108°-109° C. The preparation of the ethyl ester of thioglycollic acid is the next step and here too we followed the directions of Klason (17). The excess of alcohol is removed by distillation under diminished pressure and the ester is freed of the sulphuric acid employed in the esterification by shaking two or three times with distilled water, though some loss of material is here encountered in consequence of the partial solubility of the ester in water.

It was found that the ester readily dissolves antimony oxide and enters into combination with it, the products of the reaction being a heavy oil and a layer of water which floats on the surface of the oily or syrupy antimony compound. Quantitative experiments showed that six molecules of the ester will dissolve one molecule of Sb_2O_3 and the reaction may therefore be represented as follows:



The reaction takes place with evolution of heat. It has been our custom in the preparation of the new compound to use from 20 to 25 gms. of the ethyl ester ($\text{HS. CH}_2 \text{ COOC}_2\text{H}_5$) and to add to it in portions of half a gram or so, a little more than the calculated quantity of the oxide, say 10 gms. The mixture is well shaken

during the intervals of adding the oxide and if the heat of reaction itself is insufficient, extraneous heat may be applied to hasten the solution of the oxide. When all but a mere trace of the oxide, which is held in the form of an emulsion in the extruded water, has been dissolved, the oily antimony compound is separated and cleared by filtration.³ This compound is apparently not adapted for therapeutic purposes, as it is insoluble in water and as its solutions in oily menstrua induce pronounced local irritation. We have, therefore, changed this compound into the triamide,



To this end the antimony-ester compound is dissolved in absolute alcohol and ammonia is passed into the solution until the reaction is judged to be complete. At the first contact with ammonia the solution becomes turbid, the turbidity then gives place to a mass of slender prismatic crystals, and as the solution becomes warm these disappear and the newly formed amide settles out as a thick viscous mass at the bottom of the flask. At this point the flask is removed and a large excess of ether, which has been freed from peroxide, is added in order to throw out the amide which still remains in solution. After allowing the flask to stand for a few hours the supernatant fluid is removed and the triamide dried *in vacuo* over sulphuric acid and paraffine for the complete removal of adherent ammonia and alcohol. For purposes of analysis the compound may be further purified by solution in the smallest quantity of water, precipitated with alcohol and ether and again dried *in vacuo*. The amide as above prepared is, however, pure enough both for analytical and therapeutic purposes. When dried *in vacuo* it may be described as a colorless or slightly reddish semi-resinous compound which is soluble in water in all proportions with a neutral reaction and only slightly soluble in strong alcohol. The addition of the fixed alkalis to an aqueous solu-

³ On long standing in a tightly corked flask which is almost filled with the oil, clusters of glittering prismatic crystals separate out and a large part of the oil may be found after a few weeks to be transformed into these crystals.

tion decomposes this amide with deposition of antimony oxide and on standing exposed to light and air such solutions also deposit antimony sulphide.

In proof of the statement that the elementary composition of this compound must be represented by the formula $\text{Sb}(\text{S} \cdot \text{CH}_2\text{CONH}_2)_3$ we append the following analytical data:

I. 0.1686 gm. triamide was boiled in a Kjeldahl flask with a sufficient quantity of a dilute solution of sodium hydroxide until all of the ammonia was driven off. Hydrochloric acid was then added in slight excess and the solution was again boiled until all traces of hydrogen sulphide were removed. Sodium bicarbonate in excess was then added and the solution was titrated with an approximately $\frac{N}{10}$ solution of iodine (1 cc. = 0.0059 gm. Sb.). 8.80 cc. of this iodine solution was required for the completion of the reaction = 0.0516 gm. Sb = 30.61 per cent Sb.

II. 0.3849 gm. triamide was dissolved in water and treated with hydrogen sulphide, the Sb_2S_3 was collected, dried and found to weigh 0.1645 = 0.1175 gm. Sb. = 30.53 per cent Sb. The filtrate from the sulphide was evaporated to dryness and then analyzed for sulphur by the method of Carius, when it yielded 0.6822 gm. BaSO_4 = 0.0937 gm. S = 24.34 per cent S.

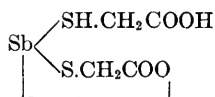
III. A nitrogen determination was made by boiling solutions of the amide with an excess of free alkali, collecting and estimating the liberated ammonia. 0.2502 gm. amide yielded a quantity of ammonia which required 18.05 cc. of $\frac{N}{10}$ acid for its neutralization = 0.02527 N. = 10.10 per cent N. This analysis shows a

SUMMARY OF ANALYSES	THEORETICAL REQUIREMENTS FOR $\text{Sb}(\text{S} \cdot \text{CH}_2\text{CO} \cdot \text{NH}_2)_3$
Sb = 30.61 per cent 30.53 per cent	Sb = 30.77 per cent
S = 24.34 per cent	S = 24.61 per cent
N = 10.10 per cent	N = 10.77 per cent

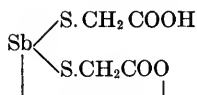
slight deficiency of nitrogen as compared with the theoretical requirements for our compound (10.77 per cent N). The deficiency is accounted for by the fact that the amide had stood for a

considerable time *in vacuo* over sulphuric acid at the time when this analysis was made. It slowly loses ammonia under these conditions, as was proved by the results of a second analysis a couple of weeks later when the nitrogen content had fallen to 9.64 per cent.

These data can leave no doubt that we are here dealing with an antimony compound of the composition above indicated. To the best of our knowledge the antimony ester $\text{Sb}(\text{S}.\text{CH}_2\text{COOC}_2\text{H}_5)_3$ and the triamide $\text{Sb}(\text{S}.\text{CH}_2\text{CONH}_2)_3$ furnish the first examples of the attachment of three radicles of thioglycollic acid to a single atom of antimony. The analogous compound of arsenic⁴ and thioglycollic acid $[\text{As}(\text{S}.\text{CH}_2\text{COOH})_3]$ is well known as having been prepared by Rosenheim and Davidsohn but the only compound hitherto known as obtainable from thioglycollic acid and antimony oxide is antimony thioglycollic acid



in which one affinity of the antimony is satisfied by a replacement of the hydrogen in the carboxyl radicle of the acid. In the course of an investigation on the formation of complex salts Rosenheim and Davidsohn (18) described a compound which they supposed to have the composition $\text{Sb}(\text{S}.\text{CH}_2\text{COOH})_3 + 12 \text{H}_2\text{O}$. The data given by these authors in support of this formula were very inadequate and unconvincing. On repeating their work, Klason and Carlson could only obtain the compound



and the proofs furnished by these investigators for the correctness

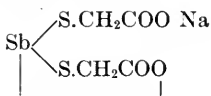
⁴ Friedberger, (Berl. Klin. Wochenschr. xiv, pp. 1714-1717, 1908) finds that a mixture of atoxyl and thioglycollic acid acquires an increase of trypanocidal power on standing and is then also effective in expelling the trypanosomes from the circulation in mice. Ehrlich mentions the compound of arsenic and thioglycollic acid, $\text{As}(\text{S}.\text{CH}_2\text{COOH})_3$, as one of a series of compounds that are still effective in cases of atoxyl-resistance. Aside from these two instances we have found no writers who have made use of thioglycollic acid or of a thioglycollate of arsenic, and their work was only brought to our notice after our own experiments with the above antimonials were nearing completion.

of this formula are entirely convincing, as they give complete elementary analyses.

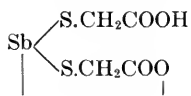
Ramberg (19) also states that he was unable to obtain the compound of Rosenheim and Davidsohn and is in agreement with Klason and Carlson that only the compound described by them can be obtained when thioglycollic acid is allowed to act upon antimony oxide. We may add that this has been our own experience also.

We would state in this connection that mercuric oxide was found to react even more readily and with greater evolution of heat than antimony oxide with the ester of thioglycollic acid. On subsequent treatment with ammonia a compound was obtained which was soluble with difficulty in water and which crystallized in the form of delicate slender prisms from its solutions in hot water. The low solubility of this compound discouraged further examination of it at this time, but we hope to continue our studies in this field in the near future. Anilides of both antimony and arseno-thioglycollic acid ($\text{As}(\text{S}.\text{CH}_2\text{COOH})_3$) were also made but as these compounds also are insoluble in water we have not studied them any further.

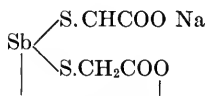
Sodium Antimony Thioglycollate.



This compound was made according to the directions of Klason and Carlson (20) and of L. Ramberg (21). For the preparation of thioglycollic it is well to proceed according to the directions given by Klason and Carlson, and for the conversion of this into the antimony compound and the preparation of the sodium salt the directions of Ramberg should be followed. This writer ascribes to this salt the formula $\text{NaC}_4\text{H}_4\text{O}_4\text{S}_2\text{Sb}$, H_2O and states that it is obtained in the form of colorless prismatic crystals which are agglomerated into thick crusts and which are extremely soluble in water. For purposes of practical therapeutics it is sufficient to proceed as follows in the preparation of this salt. The pure crystalline acid



is suspended in a sufficient quantity of water and then converted into the sodium salt



by the addition of the calculated quantity of pure sodium carbonate, the solution is then concentrated (preferably under diminished pressure) until the solution becomes a thick syrup. Absolute alcohol is now added in small portions at a time until the salt is completely precipitated. In this way a good yield of a partially crystalline white sodium salt is obtained which is sufficiently pure for all practical purposes.

Aqueous solutions are not entirely stable, although somewhat more so than those of the triamide. A trace of antimony sulphide is easily removable by filtration and it is advisable that solutions should not be allowed to stand for any length of time.

RESULTS OF TREATMENT OF RATS

Sodium antimony thioglycollate and the triamide of antimony thioglycollic acid have been used in the treatment of white rats experimentally infected with various species of trypanosomes⁵ as *T. brucei*, *T. evansi* (both surra of India and surra of Mauritius) or *T. equiperdum*. The nagana (*T. brucei*) strain used was exceedingly virulent, killing the rats in 72 to 84 hours regularly. The surra of India strain was practically just as virulent while that of Mauritius and that of dourine were much less virulent.

An attempt was made to protect rats against infection by administering sodium antimony thioglycollate 24 hours previous to the intra-peritoneal inoculation with trypanosomes. No protection at all was afforded, each of the three animals so treated developing the disease in the usual time.

⁵ It gives us great pleasure to acknowledge our indebtedness to Professor F. G. Novy of the University of Michigan for sending us rats infected with nagana; and to Dr. Simon Flexner, Director of the Rockefeller Institute for Medical Research, for supplying us with strains of surra and dourine.

TABLE I
Drug was administered at time of inoculation with the trypanosomes

DRUG GIVEN	DISEASE INFECTION	DATE OF INFECTION	NO. OF RATS INFECTED	DOSE	DATE OF TREATMENT	REMARKS
Sodium anti-mony thio-glycollate	Nagana	Jan. 11	1	mg.	Jan. 11	In good condition on July 1, (170 days).
	Nagana	Jan. 14	1	3.5	Jan. 14	Died of pneumonia on June 23, no trypanosomes could be found in the blood, two hours after death.
	Nagana	Jan. 17	2	6	Jan. 17	One of this pair died of pneumonia on the 86th day. No trypanosomes. The other is still living and is in good condition on July 1, (164 days).

TABLE II
Treatment was commenced from 18 to 24 hrs. after inoculation with nagana

DRUG GIVEN	DATE OF INFECTION	NO. INFECTED	DOSES GIVEN	EFFECT ON TRYPANOSOMES	RELAPSE	DEATH	REMARKS
Sodium anti-mony thio-glycollate	Jan. 3	3	{ 2 mg. on Jan. 4, 5, 6, 7,	disappeared	none	1 on 25th day 1 on 26th day	Accidentally crushed No T. in blood. Cause of death unknown. Living after 178 days
	Jan. 4	2	{ 1 mg. on Jan. 5, 6, 7, 8,	disappeared	none	1 on 16th day	Infection from bites of other rats Living after 177 days
	Jan. 4	2	3 mg.	disappeared	none	none	Living after 177 days Living after 177 days
	Jan. 4	2	{ 1 mg. on Jan. 5, 7, 9, 11	disappeared	none	none	Living after 177 days Living after 177 days
Triamide	Mar. 10	2	10	disappeared	none	Living after 112 days	
			8	disappeared on 12th day	none	19th day Died on 19th day	

It was found, however, that the administration of either of these drugs subcutaneously at the time of the injection of the trypanosomes was always an absolute protection against infection as will be seen by a study of Table I. At different times four rats were so treated, two of which are still living after a period of four months without having shown any signs of infection, while the third died on the 160th and the fourth died on the 86th day from pneumonia. At no time have trypanosomes been found in the blood of any of these rats.

The time elapsing between the date of inoculation and the beginning of treatment is the most important factor in determining the results obtained in treatment; the longer the period before the institution of treatment, the smaller the degree of success from the point of view of a radical cure; the shorter the period, the greater the success.

Sodium antimony thioglycollate given any time within 24 hours after intra-peritoneal inoculation furnished an absolute protection in every instance in which it was tried. Nine rats were given this drug subcutaneously in periods varying from 18 to 24 hours after infection. The trypanosomes disappeared and in no instance returned as shown in Table II. Six of these rats are still living after periods of 3 to 4 months. One was killed accidentally, while another died on the 26th day of an unknown cause, but no trypanosomes were ever found in its blood.

The triamide was tried similarly on 2 rats. One had a relapse on the 12th day and died on the 19th day, but the other is still living after 2 months and exhibits no evidence of the disease.

When the treatment is deferred until 48 hours after the infection is given, the trypanosomes in the blood are very numerous. Administration of either drug at this time will completely remove all the trypanosomes from the blood in $1\frac{1}{2}$ to 2 hours, but relapses will occur in nearly every instance unless repeated doses of the drug be administered. When a moderate dose of either preparation is given at the end of 48 hours and repeated on the 4th, 6th and 8th days after the infection, the blood remains free from trypanosomes for a period of 2, 3 or 4 weeks, and in one instance the trypanosomes never returned. This rat weighing 80 gms., received 2 mg.

of the sodium salt of antimony thioglycollate on December 23d and again on the 24th and on January 3d, 10th and 15th. The rat is still alive on July 1, 1910, after 189 days and the trypanosomes have not reappeared.

The great majority of these rats have been subjected to an intermittent treatment, the drug being administered at intervals of from 5 to 8 days. As a consequence many of these rats have shown no trypanosomes in the blood and have appeared perfectly normal over periods of months. Others have had relapses and have not been able to withstand the effects of repeated doses. Of this series of 22 rats treated with sodium antimony thioglycollate, 6 are still living; of the series of 26 rats treated with the triamide 10 are still living.

When treatment is not instituted until the end of the 3d day or until terminal convulsions are present, the blood is found to be swarming with the parasites. In many of these cases there are present in the blood half as many parasites as there are red cells. The administration of the drug even when deferred until the terminal convulsions are present, or until the animal is too weak to stand, will cause a rapid disappearance of the trypanosomes from the blood. Rats, practically moribund at the time of its administration, have been observed standing on the edge of the water bowl, drinking, within two hours after the injection of the drug, and such rats have been kept alive and free from trypanosomes for weeks before they finally succumbed.

Fourty-four rats of this series (see Tables IV, IVa and IVb) have received treatment with the sodium salt at periods of 3 days or longer following infection. These rats have all been given the intermittent treatment and some have been thus kept for periods longer than 3 months without showing a relapse. When treatment is stopped, however, relapses occur at periods of from 6 and 7 to 15 and 20 days.

A few of these rats have received one dose of mercury but it was deemed wise to try the effect of these antimony salts themselves and so the mercury treatment was not continued.

Of the 44 rats treated in this series 11 are still living, the period of survival ranging from 40 to 152 days.

TABLE III
Treatment given 48 hours after inoculation

DISEASE	DRUG GIVEN	DATE OF INFECTION	NUMBER TREATED	DOSE IN MGS.	DATES OF TREATMENTS	RELAPSE	DAY OF DEATH	REMARKS
Nagana ...	Sodium antimony thio-glycollate	Dec. 21, 09	1	3	Dec. 23-24. Jan. 3-10-15	none		Living after 191 days Found in water bowl
		Dec. 21, 09	1	2	Dec. 23-24	none	16th day	
		Jan. 1, 10	3	2	Jan. 3-5-7-9-24-27-30 Feb. 5-12-16-21-25. Mar. 4-12-18-23-28 Apr. 2-7-11-17	Relapse	30th day	Died day after last treatment
						Relapse	38th day	
						Relapse	108th day	
		Jan. 3, 10	1	1	Jan. 5-9-16-18-24-30. Feb. 3	18th day	37th day	No T. in blood at death No T. in blood at death No T. in blood at death
		Jan. 3, 10	2	1	Jan. 5-6-7-8-13-17-19-21	15th day	37th day	
						18th day	39th day	
		Jan. 17, 10	1	7	Jan. 19-24-31. Feb. 7	none	17th day	Died of the toxic effects of the drug
		Jan. 17, 10	1	6	Jan. 19-24-30. Feb. 7-12-14	none	30th day	
		Jan. 22, 10	3	5 and 6	Jan. 24-27-30. Feb. 5-14-19-24 Mar. 1 3 mgs. triamide Mar. 12	Relapse	42d day	Changed to triamide
						Relapse	49th day	
							21st day 50th day	

Nagana ...	May 18, 10	1	4	May 20	none		Living after 51 days
	Apr. 20, 10	1	2	Apr. 22-24-29. May 9-12-19	none		Living after 71 days
	Apr. 6, 10	1	4	Apr. 8-11-16-21-29. May 4-11-20			Living after 86 days
Surra of India	Feb. 25, 10	1	2	Feb. 27, 1 mg. HgCl ₂ Mar. 2	none	10th day	
	Mar. 22, 10	2	2 and 3	Mar. 24-29. Apr. 5-11-16-21-28	none	38th day	
	Mar. 12, 10	1	2	Mar. 14-16-19-22-29. Apr. 5	none	31st day	
Surra of Mauritius	Apr. 9, 10	1	2	Apr. 11-15-20-24-29. May 4-11-18-24.	none		Living after 82 days
	Apr. 20, 10	1	2	Apr. 22-24-29. May 4-12-19	none		Living after 71 days

Number still living, 6

Total number treated, 22

TABLE IIIa
Treatment given 48 hours after inoculation

DISEASE	DATE OF INFECTION	NUMBER	DOSE IN MGS.	DATES OF TREATMENT	RELEASE	DAY OF DEATH FROM DATE OF INFECTION	REMARKS
Nagana	Feb. 24	2	6 and 10	{ Feb. 26, Mar. 7, (3 mgs. HgCl ₂ 2-28 and 3-5)	none none	12th day 17th day	No T. in blood No T. in blood
	Mar. 10	3	4 and 5	{ Mar. 12-14-17-20-23-29 Apr. 5-12-18-24-30. May 6-13-18-23	May 18-23	18th day	Accidentally killed Second rat escaped. Third is living after 11 ¹ days.
	Mar. 23	1	5	{ Mar. 25-27. Apr. 8-16-21-27			
	Mar. 31	1	7	Apr. 2-5-8-13-19-25-29	none	33d day	Living after 99 days
	Mar. 10	2	5, 7 8	{ Mar. 12-15-19-23-29. Apr. 5-12-18 Apr. 24-30. May 8-16-23	none none		Living 112 days Living 112 days
	Apr. 11	1	5	{ Apr. 13-16-20-24-29. May 4-13-20	none none		Living after 80 days Died of the toxic effects of the drug.
	Apr. 4	1	5	Apr. 6-11-14-16-21	none	18th day	
	May 11	1	4	May 13-17-19	none		Living after 20 days
	Mar. 12	1	5	{ Mar. 16-19-23-29. Apr. 5-9-13	none	32d day	

Surra of India	Mar. 8	2	10	{ Mar. 10-12 (5 mgs.) 15-20-26-30 Apr. 5-12-18-24-30. May 6-12-18-24	none	5th day	Overdose
	Mar. 10	2	8 and 10	{ Mar. 12-15-19-23-29 (5 and 6 mg.) Apr. 5-11-16-22-28	relapse	56th day	Living after 114 days
	Feb. 9	1	5	{ May 5-12-19 Feb. 11-16-24. Mar. 4-10-16-23-29	none		No T. in blood Living after 112 days.
	Mar. 26	3	3	{ Apr. 5-11-17-24-30. May 6-13-19 Mar. 28-31. Apr. 5-8-13-24		35th day 24th day 29th day	Living after 141 days
	Apr. 6	1	5	{ Apr. 8-13-16-22-28. May 4-11	none	38th day	
Surra of Mauritius	Apr. 2	1	5	{ Apr. 4-11-16-21-28. May 2-11	none	45th day	
	Apr. 22	1	2	Apr. 24-30	none	11th day	
	Apr. 28	1	5	Apr. 30. May 6-31	none	34th day	
Dourine	May 11	1	5	May 13-15-19-24	none		Living after 50 days
Total number treated		26					Number still living, 10

Forty-nine rats of this series have been treated with the triamide (see Tables IVc, IVd and IVe) and of this number 20 are still living (July 1), the period of survival varying from 46 to 131 days. It would appear that of the two antimonials, triamide and sodium salt, the former is better tolerated over long periods of time.

DOSAGE

Sodium antimony thioglycollate can be administered to rats weighing 100 gms. in 5 and 6 mg. doses, while 10 mgs. can be given to a rat of 200 gms.

The triamide is less toxic, 8 to 10 mgs. being readily tolerated by a 100 gm. rat and 20 mg. by a 200-gms. rat.

These drugs may both be administered subcutaneously without evidence of irritation in 0.75 per cent NaCl solution, the solutions containing anywhere from 5 to 10 mgs. and the triamide as high as 15 mgs. to each cc. These solutions must be made up in small quantities for they do not keep well on standing. The sodium salt will remain clear at times for weeks but the triamide solution continuously precipitates antimony sulphide, which is, however, easily removed by filtration.

TREATMENT OF DOGS

Like other investigators in this field we have found dogs more susceptible to the toxic action of antimonials and arsenicals than smaller animals. It was determined by experiment that repeated doses larger than 8 mgs. pro kg. of the sodium salt or 10 to 12 mgs. pro kg. of the triamide, could not be tolerated. The toxic effects of these large doses repeated several times at intervals of a few days were an oedematous bronchopneumonia, an enteritis of the hæmorrhagic type and nephritis, especially when the treatment was prolonged. Prolonged treatment with somewhat smaller doses but too often repeated induces a type of chronic intoxication characterized by marked emaciation and fatty changes in the liver and other organs, and here too, we may meet with damages to the lungs, kidneys or gastric tract sufficient to account for death. In some cases even a single administration of the dose above named was not well tolerated.

TABLE IV
Treatment not given until the end of the third day after infection or later. Drug used, sodium antimony thioglycollate

DISEASE	DATE OF INFECTION	NUMBER TREATED	DOSE MG.	DATES OF TREATMENT	RELAPSE	NO. OF DAYS RAT LIVED	REMARKS
Nagana	Dec. 19	1	3	Dec. 22-24, Jan. 3-10	none	22	Died of pneumonia. No. T. in blood
	Jan. 3	1	4	Jan. 6-12-19-30, Feb. 4	Jan. 30	34	T. in blood
	Jan. 4	1	4	{ Jan. 7-11-15-24-30, Feb. 12-20-26, Mar. 4-7	none	65	Given Hg. on 7th
	Jan. 4	1	5	Jan. 8-11-16-24, Feb. 5-14	Feb. 21	48	T. in blood
	Jan. 8	1	3.5	Jan. 11-15-18	none	21	No T. in blood
	Jan. 8	1	5	Jan. 11-15 18-30, Feb. 7	none	39	No T. in blood
	Jan. 11	1	5	Jan. 14-18-24	none	17	No T. in blood
		1	6	Jan. 14-18-24-30	none	28	No T. in blood
		1	7	Jan. 14-30, Feb. 4-7	Jan. 30	32	No T. in blood
	Jan. 14	2	5	Jan. 17-20-24-31, Feb. 7-12-19	none	17	No T. in blood
				Jan. 12	Jan. 12	37	
	Jan. 17	2	7	{ Jan. 20-24-30, Feb. 5-12-21-26, Mar. 5-13-20-25	none	43	No T. in blood
	Jan. 25	1	8.5	Jan. 28, Feb. 1-5-14-25	several	70	No T. in blood
	Jan. 28	2	10	Died next A.M. after treatment	Jan. 14	33	No T. in blood
				{ Jan. 31, Feb. 3-7-14-21-26, Mar. 3-9-14-22	4		
					Mar. 22	57	T. in blood at time of death
	Mar. 25	1	2.5	{ Mar. 28-31, Apr. 5-8-14-19-24-31	none	39	
	Apr. 8	1	3	Apr. 11-14-20-24-28, May 4	May 13	36	Died of trypanosomiasis
	Apr. 15	1	3	Apr. 18-22	none	21	Chloroformed

TABLE IV^a

Treatment not given until the end of the third day after infection, or later. Drug used, sodium antimony thioglycollate

DISEASE	DATE OF INFECTION	NUMBER TREATED	DOSE	DATES OF TREATMENT	RELAPSE	NO. OF DAYS LIVED	REMARKS
Nagana	Apr. 6	1	4	{ Apr. 10-11 (5 mg. Triamid)-16-21-24. May 4-11-21	none		Living after 85 days
	Jan. 30	1	5	5 Mg. on Feb. 3-10	none	11	Peritonitis
	Feb. 2	1	3	Feb. 5-7-9	none	29	No T. in blood
	Feb. 9	1	3	Feb. 12-19-24. Mar. 4	none	14	
Surra of India	Feb. 18	1	7.5	Feb. 21	none	13	
	Apr. 8	1	3	Apr. 14-19-21-27. May 5-11-18	none	49	Cause of death not known
	Mar. 28	1	8	{ Mar. 31-5-(7 mg.) Apr. 9-14-18-24 (5 mg.) 29-May 5-19-24	none		Living after 94 days
	Apr. 8	1	2	Apr. 11-13-18-22-28	none	24	
	Mar. 22	1	2 and 3	Mar. 25-29. Apr. 5-11-16-21-28	none	39	
	Apr. 11	2	3	Apr. 14-21-30. May 5-11-19	none	32	
	Apr. 29	1	4	May 4	none		Living after 80 days
					none		Living after 63 days
	Jan. 26	1	2.5	Feb. 3-7-14	none	17	
	Jan. 29	1	8	{ Feb. 7-(5 mg.) 14-19-28. Mar. 12 (2.5 mg.) 16-18. (2 mg.) Apr. 5-11-15-21-29. May 6-13-20	none		Living after 152 days
Surra of Mauritius	Feb. 1	1	4	Feb. 14-19-28. Mar. 2	none	36	No T. in blood
	Feb. 18	1	5	{ Feb. 21. Mar. 4-16-25-30. Apr. 5-11	Mar. 20	56	No T. in blood

TABLE IVb
Treatment not given until the end of the third day after infection, or later. Drug used, sodium antimony thioglycollate

DISEASE	DATE OF INFECTION	NUMBER TREATED	DOSE IN MGS.	DATES OF TREATMENT	RELAPSE	NO. OF DAYS LIVED	REMARKS
Surra of Mauritius	Mar. 28	1	4	{ Apr. 2-5-8-16-21-29. May 4-13-19	none		Living after 94 days
	Feb. 18	1	5	{ Feb. 21. Mar. 4-20-25-30	none	63	
	Apr. 24	1	4	{ Apr. 5-9 Apr. 28. May 4	none		Living after 67 days
Dourine	Jan. 26	1	2	{ Feb. 9-16-24. Mar. 4-12-20-28 Apr. 5-13-21-30. May 12-19-27	{ Apr. 30 May 12, June 27, June 4	133	{ Died June 7th of Pneumonia; had been paralysed in hind legs for about 8 weeks before death. Penis diseased.
	Mar. 30	2	3	Apr. 2-5-9-28. May 6-13-20	{ none May 6	45	Living after 91 days No T. at death
	Apr. 8	1	5	May 11-15-19-24			Living after 63 days
	May 18	1	5	May 21			Living after 43 days
	May 6	1	5	May 12-15-19-25	none	21	Cause of death not known
	May 21	1	6	May 24	none		Living after 40 days

TABLE IVc

Treatment not given until the end of the third day after injection or later. Drug used, Irtamide of antimony thioglycollic acid

DISEASE	DATE OF INFECTION	NUMBER TREATED	DOSE IN MGS.	DATES OF TREATMENT	RELAPSE	NO. OF DAYS LIVED	REMARKS
Nagana	Feb. 19	1	5	{ Feb. 24 (HgCl ₂)—Mar. 7-14-19-23-29	none		Living after 131 days
	Feb. 19	1	12	{ Apr. 5-12-19-24-30. May 5-11-18-24. Feb. 25. Mar. 2(7 mg.) 7-10	none	24	No T. in blood
	Feb. 24	1	10	Mar. 1-7	none	17	No T. in blood
	Feb. 19	1	5	{ Feb. 24-Mar. 2-4-7-14-23-29 Apr. 4-12-19-24	relapse	57	
	Feb. 21	1	7	{ Mar. 10-15-19-26-31. Apr. 5-12-16-22-30. May 6-13-20	none		Living after 129 days
	Mar. 4	2	7	{ Mar. 8-14-19-27-30. Apr. 5-9-14-20-28	none	29	No T. in blood
	Mar. 28	2	7	{ May 4-11-18-23-31. June 7	none	100	Died of pneumonia
				{ Mar. 31. Apr. 5-18-13-20-24-30	May 5	77	Died of pneumonia
				{ May 5-11-15-21-28. June 4, 9	{ May 16, 28	76	Died of pneumonia
	Mar. 31	2	8-10	{ Apr. 7-11-16-21-26-30. May 5-11-16-21	Apr. 30	14	Living after 91 days
	Mar. 15	1	5	Mar. 18	May 4	10	Relapse
	Mar. 18	1	5	Mar. 21-25	none	10	Swollen face, cause of death not known No T. in blood
Surra of India	Apr. 13	1	5	Apr. 16-21-24-29. May 5-14	none	32	Living after 53 days
	May 8	1	5	May 11-17-19	none		Living after 59 days
	May 2	1	6	May 6-13-16-23	{ May 13 and 23		{ Died June 22. Penis diseased, broncho-pneumonia, no trypanosomes in blood immediately after death
	May 5	1	6	May 8-13-8-23-31. June 6-10	May 18		
	May 15	1	5	May 18-20-25	none	46	
	Feb. 25	1	3	Feb. 28-Mar. 3	none	9	No T. in blood
	Feb. 27	1	3	Mar. 2-4	none	10	No T. in blood

TABLE IVd
Treatment not given until the end of the third day after injection or later. Drug used, triamide of antimony thioglycolic acid

DISEASE	DATE OF INFECTION	NUMBER TREATED	DOSE IN MGS.	DATES OF TREATMENT	RELAPSE	NO. OF DAYS LIVED	REMARKS
Sutra of India	Mar. 2	1	5	Mar. 5-7 (3 mg. HgCl_2)-12	none	11	No T. in blood
	Mar. 5	1	5	{ Mar. 8-16-21-26. Apr. 5-13-27. May 2-8		64	No T. in Blood
	Mar. 20	1	6	{ Mar. 23-26-30. Apr. 7-13-22-28 May 4-12-20			Living after 101 days
	Mar. 23	2	3 and 5	{ Mar. 26-29. Apr. 5-9-15-21-29 May 5-11-19	none	43	Living after 109 days
	Mar. 14	1	6	{ Mar. 17-21-26-31. Apr. 5-11-16-20	Apr. 20	37	T. in Blood.
	Mar. 31	2	7 and 10	{ Apr. 3-6-(5 to 8 mg.)11-16-21-28. May 4-12-19	none none	30	Living after 91 days
	Apr. 3	2	10	Apr. 6-9-14-21-28. May 4-13		38	
	Apr. 19	1	3	{ Apr. 22-24-29. May 5-11-15-21 June 1	none May 21	47 46	Died of Pneumonia.
	May 1	1	8	May 4	none		Living after 61 days
	Feb. 23	1	5	Feb. 27. Mar. 4	none	12	
Sutra of Mauritius	Mar. 20	1	3	{ Mar. 24-28. Apr. 3-5-9-15-21-28 May 5-12-14	none		Living after 101 days
	Mar. 24	2	5	{ Mar. 28-31. Apr. 2-5-11-16-21-29 May 6-13-20	none none		Living after 97 days Living after 97 days

TABLE IVe

Treatment not given until the end of the third day after injection or later. Drug used, triamide of antimony thioglycollic acid

DISEASE	DATE OF INFECTION	NUMBER TREATED	DOSE IN MGS.	DATES OF TREATMENT	RELAPSE	NO. OF DAYS LIVED	REMARKS
Surra of Mauritius	Mar. 20	1	3	{ Mar. 24-31-Apr. 5-8-13-18-24-30, May 8-15-23			Living after 101 days
	Mar. 17	1	5	{ Mar. 20-24-29			Living after 104 days
	Apr. 6	1	5	{ Apr. 4-12-19-30			
	Apr. 14	1	5	{ May 8-15			
	Apr. 17	1	5	{ Apr. 9-13-16	none	17	Toxicity
Dourine				{ Apr. 18-23	none	17	Toxicity
				{ Apr. 20-24-29, May 4-12-19	none		Living after 75 days
	Mar. 11	1	5	{ Mar. 14-17-23-24, Apr. 5-11-16			
	Mar. 17	2	6	{ -21-27, May 2-8-15-23	none		Living after 111 days
	Mar. 14	1	20	{ Mar. 30, Apr. 5-9-14-20-28	none	38	No T. in blood
	Mar. 4	1	7	{ May 4-12-26	none		Living after 104 days
	Feb. 9	1	10	{ Mar. 17-29, 15 mgs. Apr. 5-13-20-28, May 6-14-23			Living after 107 days
	Mar. 1	1	5	{ Mar. 8-(2 mgs. HgCl ₂ 10)14-19			
	May 15	1	5	{ 25-30 (5 mgs.) Apr. 5-11-17-22			Living after 117 days
				{ 30-May 8-14-21			Paralysis. No T. in blood
				{ Feb. 21-Mar. 4-10-17-23-29	Mar. 23	28	Toxicity
				{ Mar. 4-7	none	8	Living after 46 days
				{ May 18-23	none		

We have settled on a dosage for subcutaneous administration in dogs of medium weight of 5 mgs. pro kg. of the sodium salt. The compounds used were made up so that one cc. contained this quantity. Formation of abscess and sloughing (at the point of injection) occurred occasionally, but in the great majority of cases no local reaction whatever followed the administration of either compound. The triamide is somewhat less irritant than the sodium salt, and here the dose employed ranged between 5 and 10 or more mgs. pro kg.

The nagana strain used by us was exceedingly virulent for dogs. The parasites appeared in the peripheral circulation 48 to 72 hours after intra-peritoneal inoculation and the infected animals died in from 5 to 14 days. When the parasites of surra of India or of dourine were used, a longer time elapsed before they appeared in the blood, but here also death occurred in from 12 to 15 days. The surra of Mauritius was less virulent, requiring from 2 to 3 weeks to kill dogs infected with it.

We have made but one experiment to test the efficacy of our drugs in preventing infection and that was carried out as follows: A small dose of the sodium salt was given subcutaneously at the same time with the intraperitoneal inoculation with nagana. A dose of moderate size of the same salt was given on the 5th day following and again on the 10th day. In this way the disease was entirely prevented, no trypanosomes appeared in the blood and repeated subinoculations into rats have given only negative results. This animal is still living (July 1), and is in good condition after 167 days.

When treatment is delayed until the infection is well advanced and unmistakable symptoms of the disease are present, with trypanosomes in large numbers in the peripheral circulation, complete cure has not been effected by the administration of these antimonials. Their administration is followed, to be sure, by the immediate disappearance of the parasites from the peripheral circulation and by a decided improvement in the clinical condition. The dogs become lively and are apparently normal, but unless intermittent doses be administered, relapse occurs. Infected animals treated when apparently moribund are profoundly altered and

apparently cured, but relapses are almost certain to occur. If repeated doses be administered at intervals of 7 to 10 days the relapse may be prevented but the animals will ultimately succumb to the toxic effects of the drug. This has also been the experience of others who have attempted to cure dogs with compounds of antimony (22) or of arsenic (23) when once the disease has become well established. In all, 9 dogs have been treated with the sodium salt, 4 of these being infected with nagana, the others with surra and dourine. One of these animals (with nagana) received one injection of the salt on the 6th day after inoculation, and died on the 26th day from the effects of a relapse. The others all died from the toxic effects of the drug and with one exception, none of these had had a return of the parasites in the blood. One animal of this series, weighing 5.5 kg. infected with surra of Mauritius, which was apparently cured survived for 107 days. The first injection was made on the 12th day after the inoculation and at varying intervals thereafter the dose of 64 mg. was repeated five times. The parasites disappeared from the blood and never reappeared and the animal seemed to be perfectly normal in every respect. An injection of the triamide was now tried but with the result that cerebral symptoms appeared and that the animal died in convulsions four days later.

Treatment with the triamide gave results very similar to those described above. Of this series 2 animals are still living. Table V gives the data in relation to this series.

THE TREATMENT OF RABBITS

All of our rabbits subjected to treatment were inoculated with the nagana strain elsewhere described. It kills rabbits in from 12 to 20 days when no therapeutic measures are instituted. The rabbit tolerates the antimonials here described better than the dog, toxic effects rarely following the administration of the sodium salt in doses of 10 mg. pro kg. or of the triamide in doses of 18 mg. pro kg.

In the treatment of rabbits as in the treatment of other animals, the time at which treatment is commenced is a factor of the greatest importance. One rabbit infected on January 17th and given

TABLE V.

Dogs treated with Triamidine

DISEASE	DATE OF INOCULATION	WEIGHT <i>Kg.</i>	DOSE <i>Mg.</i>	REPETITION	RELAPSE	LIVED	REMARKS
Nagana	Feb. 26	6.2	60	2	Positive	26	{Trypanosomes in blood at time of death, but toxic effects of the drug also apparent
Nagana	Apr. 5		36	5	Negative	40	{Died from the toxic effects of the drug. Cerebral symptoms pronounced
Nagana	Feb. 26	8.6	120	8	Positive	75	{Had several relapses, cause of death not certainly known
Surra of India....	Feb. 26		60		Negative	18	Died from the toxic effects of the drug
Surra of India....	Mar. 26		45		Negative	13	Died from the toxic effects of the drug
Surra of India....	Apr. 13	5	50	1	Negative		Living after 78 days
Surra of India....	Apr. 16		50	5	Negative	54	{Cause of death not known. Autopsy not made. No. trypanosomes in blood on day of death
Surra of Mauritius	Apr. 7	9.1	50	5	Negative		Living after 84 days

7 treated—2 living

10 mg. of the sodium antimony thioglycollate at the same time has given no evidence of the development of the disease although 164 days have elapsed. No trypanosomes are present in the blood and repeated subinoculations into rats have had negative results.

Another rabbit inoculated at the same time received 40 mg. of the sodium salt 24 hours later and has exactly the same history. The disease did not develop and the animal is perfectly normal 164 days later.

Five other rabbits have been under treatment. One of these died on the 54th day from an extensive ulceration. This animal apparently had no relapse. Another is still living 136 days after its infection, although it was not treated until the 12th day of the disease. This animal has received nine injections in all. No ulcerations are present though the animal has lost some in weight. No treatment has been given for 3 weeks and subinoculations into rats have proved negative in result. The accompanying table (Table VI) shows the details of these experiments.

TREATMENT OF A DONKEY

On March 12, a donkey weighing 570 pounds was inoculated subcutaneously with 1 cc. of blood from a 3-day nagana rat. The blood of the donkey remained free from trypanosomes until March 17, on which day two to four parasites could be found in each field of the microscope. The animal's temperature rose from 98° F. on the 16th to 100.6° on the morning of the 17th and 102.4° on the same afternoon, it was depressed in spirits and showed a watery discharge from its eyes and nose.

At 3.30 p.m. on the 18th it was given 0.2 gm. of sodium antimony thioglycollate intravenously.† Two hours later no trypanosomes could be discovered although several slides were examined. Its temperature gradually dropped, but on the 20th a large fluctuating tumor appeared between its front legs and this persisted for 4 or 5 days.

†We take great pleasure in acknowledging our indebtedness to Dr. F. H. Mackie, State Veterinarian of Maryland, for his generous assistance in the care and treatment of this animal.

TABLE VI
Treatment of rabbits infected with Nagana

DATE OF INFECTION	DOSE	WEIGHT KG.	TIME OF FIRST TREATMENT	QUANTITY OF DRUG ADMINISTERED SUBCUTANEOUSLY	DAYS LIVED	REMARKS, JULY 1, 1910
1-17-10	10 mg. Na salt	1.8	Same time	30 mg., three times. Last injection early in April	Living	Female, a week after giving birth to a litter of young weighs, 2.6 kg. In good condition after 164 days
1-17-10	20 mg. Na salt	3	Next day	40 mg., three times. Last injection early in April	Living Living	Male, now weighs 2.84 kg. In good condition after 164 days
2-14-10	40 mg. Triamide	2	Feb. 26	30 mg., six times at intervals of six days or more. Last dose (18 mg.) given on May 13	Living	Male, now weighs 1.73 kg. In good condition after 136 days
2-14-10	50 mg. Triamide	1.8	Feb. 26		1	Died next day. No trypanosomes in blood. Subinoculation into rats negative
3- 1-10	40 mg. Triamide	1.8	Mar. 15	40 mg., six times at intervals of a week	53	Death caused by ulceration of side penetrating into the peritoneal cavity
3-1-10	22mg. Triamide	1.4	Mar. 15		21	Died of antimony poisoning
3-1-10	35mg. Triamide	2.1	Mar. 15		18	Died of antimony poisoning

A second injection (0.5 gm.) was administered on the 22d, following which the temperature dropped to normal, where it continued to stay for 6 days. On the 28th, coincident with the appearance of trypanosomes in the blood, the temperature rose to 100.9°. Another dose (0.75 gm.) of the drug was administered which was followed by a normal temperature on the following day.

The blood now remained free from parasites for 20 days during which period two injections (0.5 and 0.45 gm.) were given and the animal appeared perfectly normal. A temperature varying from 100° to 102° was noted on two or three occasions, as may be seen by an examination of the accompanying chart. The animal was placed out in pasture for some time each day and was given a fair amount of exercise. Its appetite was good and it appeared in every way like a normal animal with the single exception that one of the hind legs became enormously swollen, being at one time nearly double the normal size. The swelling gradually subsided and one week later had entirely disappeared.

On April 18th the trypanosomes again made their appearance in the blood. A single injection (0.5 gm.) drove them out again but the temperature remained between 102° and 103° F. The parasites again appeared in the peripheral circulation 6 days later.

Another injection (0.55 gm.) again caused their disappearance and a coincident drop in temperature. Their return 8 days later was met with a rather too large dose (0.8) of the antimony preparation. Following this dose the animal appeared very sick, refused to eat and stood dejectedly in the corner and occasionally was found lying down. The breathing became very labored and difficult and on the 4th day following the last injection it died.

During the period of observation the urine was secured on two or three occasions and only the faintest trace of albumin could be detected. Up till death a fair quantity of urine was excreted and after death a full bladder was found. This sample, was however, unfortunately lost.

The blood count remained high throughout the disease, one week prior to death the leucocytes numbered 10,500 and the red cells 4,300,000 to the c.mm. During the disease the animal fell off considerably in weight but at death emaciation was not at all marked.

It will be seen from the accompanying chart that each injection successfully banished all parasites from the blood and that on many occasions the temperature following the injection became normal. The animal lived 57 days and was in good condition until the time of the last injection 3 days prior to death.

Professor G. H. Whipple kindly performed the autopsy for us and permits us to publish the following report on the condition of the various organs. It will be seen that the death of the donkey was caused by the toxic action of the antimony and that here as in the case of dogs a lower dosage must be adhered to in order to avoid a fatal outcome. Further experiments with large animals must decide whether smaller doses than those here employed will prevent the reappearance of the parasites and yet do no harm to the host.

Thus far our results with the donkey and with dogs have not differed from those obtained by investigators who have preceded us. Working with atoxyl, Uhlenhuth and Woithe (23) state that horses and dogs cannot tolerate the amounts of this drug which must be administered in order to destroy the parasites in their tissues. Moore, Nierenstein and Todd (24) were disappointed when they applied to donkeys inoculated with nagana their atoxyl-mercury treatment, a treatment which had been so successful in the case of rats. Not one of a considerable number of donkeys could be cured by their method. So too, Breinl and Nierenstein (25) found that arsenophenyglycin was not tolerated either by the donkey or the horse inoculated with nagana. Schilling and Jaffé (26) also found that the horse can not withstand the effects of this arsenical when administered intravenously or intraperitoneally in a total quantity of 0.06 gm. pro kg. in 2 days.

Holmes (27) claims that arsenious acid is a specific for surra in horses. According to this investigator, Lingard, Bruce and others who were the first to try arsenic, met with failure because they attempted a continuous treatment with small doses. Holmes states that arsenious acid should be given in full subtoxic doses at intervals and not in continuous daily dosage and this method, he declares, has led to a number of cures of surra.

Taken as a whole the literature on the trypanosomiasis of the

equidae and bovidae contains but few instances of successful treatment and it is evidently of the greatest economic importance that further advances should be made in this branch of therapeutics.

AUTOPSY REPORT BY PROFESSOR G. H. WHIPPLE

"The autopsy was performed 26 or 28 hours after death and all the viscera showed more or less post-mortem changes, particularly the intestines and the liver. The heart showed nothing of interest. Both lungs were very abnormal and presented an identical appearance which, however, was more marked in the left than in the right lung. The lung tissue in gross was a deep reddish purple color, rather flabby, but voluminous and very heavy. The bronchi contained a frothy serum. The cut section showed a very moist, purplish lung tissue with a few, indefinite areas which appeared to be consolidated, but the striking thing was the oedema and the congestion.⁶

Microscopical section showed an extreme grade of oedema. The alveoli being full of an albuminous coagulum, as well as considerable blood in the alveoli, and all the capillaries were greatly engorged with blood. There were a few indefinite, very small patches of bronchopneumonia.

The spleen was of normal size or possibly even smaller than normal. It was very flabby, and on section presented a dark purplish color which was blackened in places by the post-mortem change.

The liver was not enlarged and was very soft and flabby showing advanced post-mortem change. Microscopical section showed no fatty degeneration and apparently a little increase in pigment. Post-mortem digestion makes the picture rather obscure.

The intestines were examined, but post-mortem digestion was so advanced that examination was of practically no value.

The kidneys were large, and the surface speckled over with large and small ecchymoses. Section showed a very thick cortex streaked with the same areas of hemorrhage. The tissue was very moist and boggy feeling.

Microscopical section: There was a certain amount of chronic diffuse nephritis with increase in connective tissue in all parts of the cortex, but the striking thing was the acute condition. The interstitial tissue

⁶ Note by the authors: That antimony will cause this change in the lungs has long been known. It appears that Magendie first speaks of it as a "hépatisation pulmonaire" in his treatise entitled, *De l'influence de l'émétique sur l'homme et sur les animaux*—as we gather from Paraban's Thesis, Nancy, 1875.

everywhere was very oedematous and full of wandering cells, especially polymorphonuclear leucocytes. . . . All parts of the kidney were more or less involved in the acute change, and the nephritis was of an extreme grade.

Anatomical Diagnosis: Acute hemorrhagic nephritis; oedema, congestion and bronchopneumonia of lungs; postmortem digestion of viscera."

ON THE DEVELOPMENT OF RESISTANCE IN TRYPANOSOMES TO ANTIMONY THIOLYCOLLATE

That trypanosomes may develop a pronounced and an inheritable resistance to certain drugs was first clearly established in Ehrlich's laboratory in the researches of Franke and Röhl (28) on trypan red. Since then it has been shown by Ehrlich and his pupils and by other investigators that resistance is developed in the course of treatment with various arsenicals and dyestuffs, as trypan red and trypan blue, and lately Kopke (29) has described a case of sleeping sickness in which the trypanosomes were no longer affected by atoxyl.

Mesnil and Brimont (30) have observed that a surra of Mauritius strain which was atoxyl resistant in mice, rapidly acquired a similar tolerance for tartar emetic. The 5th injection of tartar emetic into mice infected with this strain was without effect on the parasites and when these were transferred to other mice they were found to retain their resistance to the drug. Even when the drug was given in the highest possible dose (0.40 to 0.45 mg. to 20 gms. of body weight) it had not the slightest influence on the course of the infection.

The antimonials used by us do not readily cause the appearance of a drug-resistant strain. We have hitherto failed entirely in our efforts to obtain a nagana strain which should be resistant to thioglycollates of antimony. We note also that Mesnil and Brimont were less successful in obtaining antimony-resistance with nagana than with surra and that here they began their work with a strain which was already resistant to atoxyl. Even when in a few cases of nagana in mice the parasites were no longer destroyed

TABLE VII
Experiments to obtain an Antimony thioglycollate-resistant strain in rats

DRUG	DATE OF INFECTION	NUMBER TREATED	DOSE IN mgs.	DATES OF TREATMENT	NUMBER OF RELAPSES	NUMBER OF DAYS RAT LIVED	REMARKS
Sodium Antimony Thioglycollate	Apr. 1	2	5	{ Apr. 4-7-11-16-21-25-30. May 5-11-16-21	3	(1) 15	One living after 90 days
	Mar. 13	2	2-5	{ Mar. 17-25-28-Apr. 5-8-15-21-28-May 4-13-20	one Mar. 28	(1) 20	One living after 109 days
	Mar. 17	1	3	Mar. 22-28, Apr. 7	none	20	
	Apr. 9	1	5	Apr. 13-16-21	none	12	Death due to the toxic effects of the drug
	Apr. 17	1	2	Apr. 22-24-25, May 5-11	one May 5	25	
Triamide of Antimony Thioglycollate acid	Apr. 4	1	5	Apr. 7-9-15-18-24-30 May 13-23	one May 23	30	Living after 54 days
	Apr. 7	1	5	Apr. 9-13-18-24			

TABLE VIIa
Experiments to produce resistance in T. brucei after passage through a donkey

DRUG	DATE OF INFECTION	NUMBER TREATED	DOSE IN mgs.	DATES OF TREATMENT	NUMBER OF RELAPSES	NUMBER OF DAYS RAT LIVED	REMARKS
Sodium Antimony Thioglycollate	Apr. 27	1	5	May 3-9-11-16-19-24	5		Living after 64 days
	May 9	1	5	May 11-20	1		
	May 11	1	3	May 14-20	2	13	Living after 53 days
	May 14	1	5	May 16-20			Killed by other rats
	May 16	1	7	May 19-21		9	
	May 19	1	5	May 21			

by tartar emetic, these authors observed that on transference to fresh mice the strain again showed its former sensitiveness to the drug.

The following instance will illustrate the difficulty of obtaining a strain which is resistant to the antimonials employed by us. A rat infected with nagana was subjected to symptomatic treatment, a small dose of sodium antimony thioglycollate being administered at each reappearance of the parasites. In this way the rat was kept alive for 108 days and died on the day following the last injection of the antimony, being apparently worn out by the combined effects of the repeated injections and the recurrences of the disease. No sign of drug resistance could be detected, although the rat had received 19 injections in all, the trypanosomes always disappeared promptly after each injection of the drug and the intervals between the relapses did not become shorter, the blood being free from trypanosomes for 6 days prior to the last injection (a period somewhat longer than the usual interval). A further attempt was made to develop resistance by transfer of the trypanosomes from this rat to others. At various times nine rats were inoculated with this strain and treated as before. No resistance has developed. Three of these rats are still living and in them the parasites respond to treatment as readily as in rats inoculated with the untreated strain of nagana.

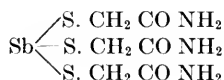
It will be seen, therefore, that we have been unable thus far to secure a strain resistant to our antimonials in cases in which rats alone have been used as animals of passage during the time of treatment.

The strain of nagana which was recovered from the donkey on its last relapse and which had been subjected in that animal to intermittent treatment with our antimonials has, however, shown some evidences of a greater resistance to antimony. Table VIIa contains the data relating to this strain and an examination of it will show that relapses occur somewhat earlier and are more numerous than with the untreated nagana strain. In the case of one rat (rat of May 11th) in this series a point was reached in which the second injection of the antimonial was powerless to free the blood of parasites. Inoculation of a fresh rat with blood from the

heart of this rat failed to infect, so that we can not know whether the hereditary characteristic had been acquired.⁷ Our work on this point may be summed up in the statement that resistance has not been noted in the prolonged treatment of any *single* species of animals infected with nagana and that the only phenomena suggesting tolerance have been encountered in the strain which was subjected to treatment *in two different hosts, the donkey and the rat.*

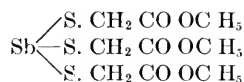
CONCLUSIONS

1. A new compound of antimony, the triamide of antimony thioglycollic acid,



has been prepared and found to be well adapted for subcutaneous and intravenous injections under the conditions stated in the body of this paper.

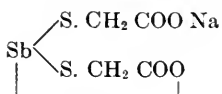
2. A new antimony-thioglycollic ester, having the composition represented by the formula,



has also been prepared, but this substance appears to be little suited to therapeutic purposes on account of its low solubility in water.

⁷Note under date of July 1. Since the above table was completed we have again found a rat inoculated with this donkey strain in whose case our sodium salt was unable, in a second dose, to drive the parasites from the blood. Subinoculation was effective in this case, *but no evidence of increased resistance could be found in the rats of passage.* Our results with rats are therefore to be placed by the side of those obtained by Mesnil and Brimont with mice, in which case also an occasional resistance did not become hereditary.

3. Sodium antimony thioglycollate,



was prepared according to the directions of Klason and Carlson and of Ramberg and was found to be well adapted for therapeutic purposes under the conditions stated in our paper.

4. This salt and the above named triamide have been tested in regard to their efficacy as trypanocidal agents in several series of inoculated rats, rabbits and dogs. The trypanosomes employed were highly virulent strains of *T. brucei* and *T. evansi* and somewhat less virulent strains of *T. equiperdum* and *T. evansi* (surra of Mauritius). Only one large animal, a donkey, inoculated with *T. brucei* was subjected to treatment.

Our results in the way of treatment compare so favorably with those obtained by others in the use of the antimonials or arsenicals of the day in experimental trypanosomiasis that a trial of the antimony thioglycollates in human trypanosomiasis and in the trypanosomic diseases of the larger animals would seem to be justified. In the case of the equidae and bovidae these drugs may be found to be of service as prophylactic agents during periods of exposure to infection in traversing a "fly region." Their relatively low cost and their small ability to induce drug-resistance in trypanosomes are factors worthy of consideration in this connection. It is not claimed that these antimonials are more efficacious in the long run as therapeutic agents than those hitherto used, but it is certainly worth while to enlarge the number of trypanocidal drugs, especially if their toxicity is less marked than that of their predecessors or if they are better adapted for subcutaneous injection.

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CHART I



FURTHER OBSERVATIONS ON THE IMMUNISATION OF ANIMALS TO THE POISONS IN FUNGI

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It has previously been pointed out (1) that it is possible to immunise animals to haemolytic extracts of the poisonous fungus *Amanita phalloides*, and that in the course of this immunisation the animals develop sera which have both antihaemolytic and antitoxic properties. The antihaemolytic strength of such sera is frequently 1-1000 or more but the antitoxic value is always low, one cubic centimeter neutralising but three or four fatal doses of the poisonous extract. At that period of our investigations upon fungi, it was believed on the basis of Kobert's earlier publications (2) that the blood-laking substance in the "deadly amanita" was the active principle, and that the problem of obtaining an antitoxic serum of a higher potency lay in the immunisation of larger animals and in the production of more powerful antihaemolytic sera. When, however, the attempts which were made to immunise goats and horses demonstrated that the treatment could be pushed only to a certain point and that the animals succumbed to large doses, it was surmised that the production by animals of an antihaemolytic serum was no sure proof that they would also produce an antitoxic serum and the suspicion arose that there might be other factors concerned than those first considered. A further study of the action of the fungi demonstrated (3) that in addition to the blood-dissolving substance, which is destroyed by heating to 60-65° Centigrade there is also present in *Amanita phalloides* a heat-resistant poison to which we gave the name *Amanita-toxin*. An examination of the fungus

extract by chemical methods was now undertaken and it has been shown by Abel and myself (4) that the two poisons may be separated by alcohol, basic lead acetate and other reagents, and that the *Amanita-haemolysin* solutions when freed of proteid give the reactions of a glucoside containing a pentose. We have subsequently developed a method for the isolation of this body and on a number of occasions have obtained highly active products which were of a sufficient degree of purity to permit preliminary analysis (5). Our final material, still highly haemolytic, gave the same reactions as those originally ascribed to it, namely, those of a pentose-containing glucoside, while its analysis gave a percentage composition of C = 48.93, H = 6.08, N = 10.83, S = 1.94, O = 32.322. We could only conclude therefore that the *Amanita-haemolysin* must belong to the group of haemolytic glucosides of which we have a number of examples in saponin, sapotoxin, solanin, etc., and not to the group of haemolytic proteids. More knowledge of the constitution of this complex body may reveal to us that particular group in its molecule which effects the solution of the red blood cells.

It has also been shown in association with Schlesinger (6) and with Prouty (7) that the *Amanita-toxin* may be isolated by the use of phosphotungstic acid (10 per cent phosphotungstic acid in 5 per cent sulphuric acid) and that when freed from impurities, it gives neither proteid, alkaloidal, glucosidal nor conjugate sulphate reactions. A further study of this poison, to determine its more exact chemical characterisation, is now in progress. Since the *Amanita-toxin* is resistant to the temperature to which these poisonous fungi may be submitted without losing their deadly properties, and is of extreme toxicity for a great variety of animals, it is believed on these and on other grounds (8) that it and not the haemolytic body is the active principle. It was moreover suspected that the presence of this poison in extracts of the plant might be the factor which prevented the immunisation of animals to such a degree that their sera contained efficient antitoxic as well as antihæmolytic substances. At the same time the fact that the haemolysin apparently belonged to the group of glucosides proved to be of great theoretical interest in

the subject of immunity and a further study of the action upon animals of the chemically separated poisons was therefore undertaken.

The relative amount of haemolysin and toxin in different extracts of *Amanita-phalloides* varies greatly, or rather, since the former body deteriorates rapidly both in the dried plant and in solution, the amount of active blood-laking material is greater in fresh than in old preparations although the absolute quantity of the glucoside may be the same in the two cases. Since both the haemolysin and the toxin kill animals, the extent to which the action of any particular preparation must be referred to either ingredient will depend upon the age of the fungi employed and the length of time the poisons have been in solution. Fresh extracts which contain powerful haemolysins will owe more of their toxicity to the activity of this body than will old ones in which the glucoside has partially lost its strength while the toxin remains unimpaired. In the same way a serum which has a powerful anti-haemolytic and a low antitoxic action will neutralise fresh far better than old extracts. Upon animals the action of the two poisons differs radically. The toxin kills acutely, the animals dying in 24-48 hours and showing no changes beyond a fatty degeneration of the internal organs. The haemolysin kills slowly in 3-10 days, the animals apparently succumbing to the extensive blood destruction. At the site of inoculation is found a huge gelatinous oedema, there is frequently a blood stained serous exudate in the peritoneal cavity, and the urine in the bladder is reddish in color but shows no intact blood cells, a true haemoglobinuria. On microscopic examination the pigment in the various organs, especially in the spleen and liver, is greatly increased. The immunisation of animals with these individual poisons, freed as far as possible from foreign admixture, has been undertaken at various times and the properties of a number of antisera have been investigated. Another glucoside found in fungi, in this case an agglutinin, has also been studied in the same connection. The results obtained with these various poisons may be briefly summarised.

THE AMANITA-HAEMOLYSIN

Animals treated with whole extracts of *Amanita phalloides* develop antihaemolytic sera which may have a strength of 1-1000, 1-2000, or even more. Such sera are obtained from rabbits after 6-8 weeks treatment. Similar sera are produced by large animals, and some time ago Dr. Kinyoun immunised a horse for me obtaining an antiserum in which 1 cubic centimeter neutralised four fatal doses of the whole extract for a 500-gram guinea pig. This serum despite its low antitoxic value was powerfully antihaemolytic and tests have been made with it now for nearly four years. When first examined it had a strength of over 1-1000, 1 cubic centimeter of a 1-1000 dilution completely neutralising a *haemolytic unit*, that is, the amount of the glucoside which will give solution of 1 cubic centimeter of a 5 per cent blood suspension in 18 hours. The strength of this serum remained almost stationary for a long period of time, but recently it has shown considerable deterioration. When such sera are precipitated by ethyl alcohol, the filtrate shows no antihæmolytic action, while a saline solution of the precipitate neutralises the glucoside to the same extent that the original serum does. This antibody is therefore either directly precipitable by ethyl alcohol, or it is so tied to the proteid fraction of the serum as to be carried down with it. In the same way it may be precipitated by basic lead acetate.

If extracts of *Amanita phalloides* be concentrated and treated with ethyl alcohol, or if the dilute solution of the plant juice be treated with basic lead acetate, the haemolysin may be obtained free from the toxin. If the fungi be first extracted with alcohol to dissolve out the toxin an aqueous solution of the residue will be found to contain the glucoside, together with a considerable amount of toxic material which may be removed by dialysis. *Toxin-free* haemolysins may thus be prepared and their strength accurately determined. Animals treated with small doses of this material quickly develop an immunity to its poisonous action. At first a little oedema develops at the site of inoculation and the animals lose in weight, but the oedema soon disappears and the weight

is gradually restored to normal. Such animals produce an effective antiserum and they are moreover absolutely immune, recovering completely from the treatment and showing no late consequences. Animals so immunised have been bled, usually after their sera show an antihæmolytic strength of about 1-1000, this being considered a satisfactory serum to study.

Finally since some doubt has been expressed as to the possibility of our antihæmolysin being in reality an antibody to a glucoside, the small amount of plant proteid in our original solutions being supposed to exert some influence upon the reaction we have described, hæmolysins have been prepared both *toxin-free* and *proteid-free* and the treatment of animals undertaken with such preparations. These hæmolysins have either been freed of proteid by uranyl acetate in alkaline solution and from the toxin by precipitation with alcohol or basic lead acetate or they have been obtained from the fungi by aqueous solution after the toxin has been taken out with alcohol. In the latter case the proteid has been removed in the usual way and the solutions then dialysed for 24-48 hours to rid them of the salts and the toxin residue. The immunisation of animals with this proteid-free solution of our hæmolytic glucoside proceeds exactly as the immunisation of animals to the toxin-free hæmolysins just described. The animals first develop a sub-cutaneous oedema and suffer a loss in weight but the swelling of the tissues at the point of inoculation disappears as the treatment is continued and the weight soon becomes normal. After 6-8 weeks treatment the serum of these animals is found to be powerfully antihæmolytic, the animals having responded to the introduction of this *proteid-free* material and having produced a characteristic neutralising serum. Thus a number of rabbits were immunised with proteid-free hæmolysins in 1909. They developed typical antihæmolytic sera; in one instance, a serum having a strength of 1-1000 and in another, a value of 1-800. These sera subsequently deteriorated in strength, when last tested having a potency of about 1-200. As was to be expected, the proteid-free hæmolysins have the same action as the solutions containing traces of plant proteid. This latter substance seems to be quite inert, exerting no influence upon

the response of the animals to the actively haemolytic glucoside. Some of the rabbits immunised to this proteid-free body were kept under observation for long periods of time to determine whether the immunity induced in them is characteristic in the sense that they do not develop late lesions and die of a chronic form of intoxication. In two instances rabbits were watched for over a year during which time they remained in perfect health. After the lapse of 7-8 months the samples of serum removed from the ear vein were found to be almost devoid of antihaemolytic action. One of these animals was subsequently killed by a dose of haemolysin and toxin while the other was employed for immunisation with another lot of proteid-free haemolysin and developed a serum of the strength of 1-400. This animal was purposely destroyed 18 months after the original treatment. The artificial immunity induced in animals by small doses of this haemolytic glucoside seems to correspond in all essential particulars to the artificial immunity produced by the true toxins of bacterial origin.

THE AMANITA-TOXIN

Since the *Amanita-toxin* is apparently the active principle of *Amanita phalloides* or at least is the more important of the two poisons in human intoxications, the haemolysin acting possibly only when poisoning follows consumption of the raw fungus, it is important to determine how far animals can be immunised to the pure toxin, free from admixture with the blood-laking material. Thus far no success has been met with in this attempt. Animals will withstand the introduction of low multiples of a fatal dose and apparently have a heightened resistance to the action of the poison but in no instance has a definite artificial immunity been established. It is evident that it was the presence of this powerful toxin in our original extracts which was the source of the discrepancy already referred to, namely that a powerful antihaemolytic serum may show but a low degree of antitoxic action, and that immune animals succumb to the introduction of high multiples of a fatal dose even when their serum is endowed with neutralising substances for the haemolytic glucoside. A curative

serum for this variety of fungus intoxication can thus be prepared to the same degree that the haemolysin acts as an etiological agent, unless further investigation shall prove that active immunity towards the *Amanita-toxin* can be brought about by some other methods.

THE MUSCARIA-AGGLUTININ

Amanita muscaria, the "yellow or fly agaric" contains a heat-resistant agglutinin which may be isolated from infusions of the plant by chemical methods and which also gives the reactions of a glucoside (9). It is apparently the first substance with this action upon blood corpuscles which has been shown to belong to this group. This agglutinin is seemingly devoid of toxic action upon animals, and large quantities can be administered subcutaneously without appreciable effect. In the animals thus far studied, where the substance was given in small doses over a period of 6-8 weeks the serum showed no increase of antiagglutinating action, inhibiting the agglutination of the corpuscles by this agent to practically the same degree as normal serum.

CONCLUSIONS

Three different substances in fungi, the *Amanita-haemolysin*, the *Amanita-toxin* and the *Muscaria-agglutinin*, have now been tested at some length in regard to their power of stimulating animals to antibody formation. But one of these, the *Amanita-haemolysin* can be said to act like a true toxin in this respect, but with this poison the immunisation of animals has now been carried out on so many different occasions and the sera produced have such definite and lasting antihaemolytic properties as to leave little doubt of the definiteness of the reaction. The fact that our chemical investigations indicate that this haemolysin must be classed as a glucoside, and that animals may be immunised to it after it has been freed of proteid, raises important questions in regard to immunity production, and suggests that the study of other substances than the toxic proteids may throw

some light upon that remarkable phenomenon in which the tissues and cells of the animal organism throw out protective substances when certain poisons come in contact with them, but fail to react in this way under the influence of other poisons.

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EXPECTORANTS

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No therapeutic group of drug-stuffs suffers so greatly perhaps from lack of exact methods of examination and careful observation as the expectorants. Consequently the few facts which the authors have gathered are presented, though they feel that they have by no means exhausted the subject and hope to continue their investigation, which force of circumstances has for the time interrupted.

There seem to be only two papers which deal experimentally with this subject. The first is by Rossbach (5) published 1882. The method he employed was the observation with the naked eye of the exposed tracheal mucous membrane in cats and dogs. The surface of the epithelium was dried with blotting paper and the time noted before it again became covered with mucus. The conclusions that he draws from his experiments may be summarised as follows: 1st, The intravenous administration of sodium carbonate led to a decrease in mucus production: the same seemed to be true of ammonium carbonate. 2nd, Local application of a 0.5 per cent solution of sodium carbonate produced no noticeable effect, while dilute solutions of ammonia or of acetic acid similarly applied brought about marked secretion. 3rd, Pilocarpine given intravenously caused a marked secretion; less marked but still abundant secretion was brought about by the intravenous administration of apomorphine and emetine. Rossbach considers the action of these three latter drugs to be peripheral either on local ganglia, nerve endings or the gland cells themselves. The second paper is by Calvert, (2) whose attention was drawn to the dis-

crepancy between the experimental results of Rossbach and the successful clinical use of alkalies as expectorants in medicine. In consequence using the same method as Rossbach he repeated with great care some of the former's experiments. He concluded that intravenous administration of sodium carbonate does cause an increase in secretion, thus contradicting Rossbach. The first of the three experiments considered by him to be positive would seem to an unbiased observer to be at the least doubtful. In other experiments he finds that intravenous injection of iodides increases tracheal secretion. Intravenous administration of emetine increases the secretion; while saponin in small doses does not increase and in large doses decreases owing to its deleterious heart action.

Neither of these papers were known to us until we had concluded our experiments. The method that they employed we had, however, tested and abandoned for two reasons. Firstly, because the liability to subjective error was so great and secondly because the appearance of secretion on any area of trachea is due to two factors, the secretion of the glands in that area and the secretion brought to the area by the cilia of the areas below. The observations on the movements of mucus by the cilia, reported in a subsequent paragraph, establish how irregular this may be under the experimental conditions employed by these observers and make it quite impossible to judge of the rate of secretion by the method they employ. The point in dispute between these two observers seems to us to be one of not great importance as sodium carbonate is never given intravenously as an expectorant and we have no reason for assuming that the small doses used as expectorants could have any marked effect upon the alkalinity of the blood. The experiments reported in this paper on ammonium carbonate, taken in conjunction with those in a previous paper by one of us upon salivary secretion (4) show how much more important the reflex secretion is than that produced by direct action upon the centres. The experiments reported in this paper and in the previous paper just alluded to show also that emetic drugs even in doses insufficient to cause vomiting do cause a marked reflex secretion. The emetic effect of sodium carbonate is so

well known, that the entire explanation of its expectorant action can be deduced from it.

The trachea and the bronchi are very richly supplied with mucous glands. In the cat these are found even when the cartilage has almost completely disappeared from the bronchi. Mucous epithelial cells seem also to occur. When we examine a series of sections of tracheae and bronchi and realize the wealth of glandular material, it seems remarkable that persons in normal health do not notice more the activity of these glands. They must produce but a small quantity of mucus, as normal persons do not find it necessary to cough or hawk to get rid in the morning of mucus which has accumulated in the pharynx.

Mucus excreted by the bronchial glands is carried very rapidly upwards by the action of the cilia. Before von Gebhardt's (3) interesting paper appeared we had carried out a series of experiments to ascertain how quickly mucus might be expected to be carried up the bronchi and trachea to the larynx. In experiment XVI of Gebhardt, undertaken 2 minutes after the dog's death, powder placed upon the opened trachea travelled at the rate of 5.3 cm. per minute (320 cm. in 60 minutes). In this case the trachea was moistened with Ringer's solution through which air had been passed. In other observations a rate of about 1-2.2-3.2 cm. per minute seemed more common. Our observations were also made on the trachea but during the life of the animal. It was observed that dust, even if fine, did not travel so rapidly as mucus which was stained by dropping into the trachea fine droplets of a solution of indigo-carmin. In several experiments the indigo-carmin was injected through the trachea by means of a fine syringe needle and the time noted when it appeared at the larynx. A fine canula inserted low down through the wall of the trachea served to deliver air from a respiration pump when necessary. The fastest rate observed was 4 cm. in 65 seconds (experiment XVI); the average seemed to be 1.5-2 cm. per minute. Judged by the movements of dust or stained mucus, the cilia appeared to be greatly affected by the thickness and viscosity of the layer of mucus covering them. We noted also that dust or stained mucus very frequently did not travel in a straight line but tended to curve to one side or the

other. Streaks of stain were very commonly seen and Gebhardt seems correct in his conclusion that not all the cilia in the trachea or on any ring are working at the same rate. The data furnished by these experiments showed that mucus due to any stimulation should reach the larynx within 10–20 minutes. Several experiments were made on the effects of moistening the surface of the trachea with saliva to which had been added various expectorants. In no case could a definite action of the expectorant be detected. The most extensive series is reported in experiment XLII.

Several methods of collecting the secretion from the bronchi and trachea were tried. The one most extensively used was the following. The animal (cats were almost exclusively used) was anaesthetised with chloroform (C) or ether (E), or both, which were followed by urethane for the period of observation. One short limb of a thin-walled glass Y-piece was tied into the upper end of the trachea, the second short limb connected by a short piece of rubber tubing to one side of a calcium chloride U-tube or was inserted directly through the cork in the top of the U-tube. The third limb of the Y-piece was connected to the blast side of a Meyer's respiration pump, to the exhaust cylinder of which the other side of the U-tube was attached. The animal was placed upon its belly with its body on an incline, so that there was a sharp slope in the trachea and in its connections to the U-tube, so that gravity aided the flow of the mucus after it passed into the canula. With this method not only the fluid mucus but any water evaporated from the mucus and also the water evaporated from the lungs was estimated. The water from this latter source varied greatly with the temperature of the animal, the amount of air passing into the lung and also probably with changes in the animal's circulation. In consequence it was found necessary to observe carefully the animal's temperature, to place an accurate governor on the pump and note that no marked changes took place in the animal's circulation. This imposed very great difficulties which were not in every case overcome: only successful cases are reported. Errors from these sources were also in part avoided by the considerable length of time (10–20 minutes) over which each observation was extended.

The bronchial-gland centre seems to be almost as easily disturbed as the salivary centre, and as the action of many of the expectorants are reflex this again caused much trouble. Our experiments seemed to show a parallelism between the activity of the bronchial centre and that for the salivary glands.

The experiments carried out with potassium iodide yielded results similar to those on the submaxillary gland. The presence of this drug in the blood stream was not sufficient in itself to set up bronchial secretion. It is excreted by the bronchial glands, experiments VI, XI, XV. If it acts as an expectorant, its action must be reflex from the stomach or mouth as in experiment 27 in the paper in "Salivary Secretion." (4) No action was detected upon the cilia. Our results are directly opposed to those of Calvert, who thought that intravenous administration of iodides increased the bronchial secretion.

If the centre was depressed as in experiment XXXVIII ammonium salts even in large doses failed to bring about increased secretion. If, however, the centre was active, secretion was brought about. In experiment XXXVI a dose equivalent to 184 gr. of ammonium carbonate for a man of 132 lbs., given intravenously, induced a marked increase of secretion. It is to be remembered that the animal was under an anaesthetic which must have greatly decreased the irritability of the gland-centre. A very good flow was caused by two and one half times this dose per os. and when it is recalled that according to Saleski and Biedl and Winterberg (1) one-half to four-fifths of the ammonium absorbed from the intestine will disappear in the liver, the effectiveness of this dose must be ascribed in part at least to a reflex action. A relatively smaller dose given intravenously caused an equally marked effect in experiment XV. Biedl and Winterberg showed that in unanaesthetised dogs symptoms referable to the central action of ammonium, occurred when enough ammonium was injected to increase its amount in the blood from a normal of about 1.5 mgm. per 100 g. of blood to 2-2.5 mgm. In a dog weighing 8.7 k. this required, in their experiment 8, 0.15 g. NH_3 (approximately 0.45 g. of ammonium chloride), the injection lasted eight minutes. Man has normally about 0.9 mgm. NH_3 per 100 g.

blood and if it had to be increased by the same amount 0.5 mgm. per 100 g. to produce a central effect then a man of 65 kilos would require 3.49 g. Now the therapeutic dose recommended is much smaller than this and as a part of that absorbed disappears in the liver, it can hardly be suggested that any action can occur on the centres. Its action must be reflex. In only one case where ammonium salts were given per os, experiment XXXIX, did we obtain a result which was slow in developing as though due to an action of the ammonium after absorption. In all other cases the rise occurred promptly, if it occurred at all. One would not expect that the reflex action of ammonium salts could be long sustained.

Ammonium chloride was found by Biedl and Winterberg to be less toxic than the carbonate when given intravenously and it is odd that experiment XXXVI so closely confirms this. Experiment XXXIX shows an action which may in part be reflex or may be on the centre.

Ipecacuanha, given as either the wine or the fluid extract (experiments XV, XXXVII and XXXVIII) was found to bring about secretion, when given per os, if the centre was active. If it was depressed (the lack of salivary flow was taken as an evidence of this), no effect was obtained. When given intravenously as emetine for example, experiment XL, a flow was at times obtained, but as a rule the disturbance of the circulation was so marked as completely to obscure this action.

Antimony was also found an effective reflex agent as may be seen in such a protocol as that of experiment XLI.

Apomorphine acts on the bronchial centre and has probably no action on the glands, as was stated by Roszbach.⁽⁵⁾ In no experiment was an action obtained when the centre was depressed, and it was difficult to obtain striking results even with the centre intact, as disturbances in circulation were so common. However, thick bronchial mucus in some cases appeared in the canula and this was taken as an indication of the action of apomorphine although the increase in weight of the absorption tube was not much greater than that of its controls. In no case when the center was depressed did mucus appear in the canula in the way

just referred to. In the paper upon "Salivary Secretion" it was shown that apomorphine had no peripheral action on the salivary gland.

Senega, given per os as the tincture, causes a good flow, as is shown by experiment XXXVIII.

Pilocarpine increases bronchial secretion, even if the centre is depressed, and atropine inhibits the flow when thus produced. Experiment XXXVI shows characteristically the pilocarpine increase and also the effect of atropine.

With the exception of experiment XXXIX, all increases in secretion which were observed after administering an expectorant per os occurred within the first 10-20 minutes and in no case did the increase continue for a long period. The animals were of course anaesthetised and it is quite possible that more prolonged reflex stimulation is obtained under normal conditions. Even a temporary increase in secretion might therapeutically be of service. In the light of our experiments it hardly seems probable that iodides can after absorption increase bronchial excretion even under normal conditions unless they do so reflexly. In the paper on "Salivary Secretion" an experiment (number 27) was reported in which iodides given per os brought about a prompt flow evidently reflex in character. Nor does it seem probable that ammonium salts act after absorption, their action in therapeutic doses must be reflex. The protocol of experiment XLII is given as an example of experiments which show that even were small amounts of ammonium chloride or carbonate or potassium iodide excreted they would have no effect upon ciliary movement and the flow of mucus.

CONCLUSIONS

If iodides produce an increase in bronchial secretion, it must be brought about reflexly.

Ammonium compounds increase secretion reflexly and possibly to a limited extent by an action on the bronchial gland centre if very large doses are given.

Antimony and ipecac and senega produce bronchial secretion reflexly. Emetine has a central action as well.

Apomorphine stimulates the bronchial gland centre.

Pilocarpine stimulates the bronchial glands peripherally and atropine depresses them.

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Experiment VI. Cat, 2 K. urethane 2 g. and high pithed, observed 20 minutes before beginning experiment.

12.22 p.m.	0.4380 g. in 20 minutes.
12.26	mgm. potassium iodide intravenously
to 12.42	0.4200 g. in 20 minutes, a failure owing to a faulty junction.
1.19	20 mgm. ammonium chloride intravenously.
to 1.39	0.4142 g. no saliva noted.
to 1.59	0.4168 g.

Centre depressed, neither potassium iodide intravenously nor ammonium chloride produce a secretion.

Experiment XI. Cat, female, 4 K., E. C. 5 g. urethane. Had been under observation for an hour, during which time ammonium chloride had been given intravenously and had caused an increased secretion but the experiment was not considered satisfactory.

to 2.37 p.m.	0.4458 in 20 minutes.
to 2.43	20 mgm. potassium iodide intravenously.
to 2.57	0.4168 the pump was at this time not regulated and ran during this period a beat slower per minute than previously.
to 3.17	0.4164
to 3.27	0.4262
at 3.35	20 mgm. potassium iodide intravenously.
to 3.57	0.4110

Potassium iodide intravenously without effect. Subsequently an increase in the amount of secretion was obtained with pilocarpine.

Experiment XV. Cat, female 2.2 K. Chloroform urethane 2.25 g. Cat had been observed for nearly an hour during which difficulty with pump and absorption apparatus prevented exact results.

to 11.05 p.m.	0.3096 g. in 20 minutes.
11.12	40 mgm. potassium iodide intravenously.
to 11.25	0.2992
to 11.45	0.2964
at 11.46	40 mgm. ammonium chloride intravenously: saliva appeared.
to 12.05	0.4156

to 12.25 p.m.	0.3400
to 12.45	0.3170
to 1.25	0.2718 in 20 minutes (suspected to be low).
to 1.45	0.3036
to 2.05	failure
at 2.06	tincture of squill 1 cc. per os; saliva appeared.
to 2.25	0.5708
to 2.45	0.3168
to 3.05	0.3080
at 3.06	2 cc. wine of ipecac per os.
to 3.25	0.3284
to 3.45	0.2634
to 4.05	0.3058

Potassium iodide without effect. Ammonium chloride intravenously gave good increase as did tincture of squill per os. Slight effect from wine of ipecac per os.

Experiment XVI Cat; high pithed; natural respiration. A minute drop of indigo-carmin was placed upon the inner surface of the trachea with a hypodermic needle. It reached the larynx in 4 minutes, distance 6 cm. 1.5 cm. per minute. The trachea was then split. Chalk placed upon the trachea travelled in one case 4 cm. in 65 seconds, in another 2 cm. in 30 seconds; these were however, much faster than what appeared to be normal, which was 2 cm. per minute.

Experiment XVII. Cat, urethane. Indigo-carmin injected into the trachea reached the larynx, 5 cm. distant, in 3 minutes. It was distributed in two long streaks when the trachea was opened.

Experiment XXXVI. Cat, female, 2 K. Ether and chloroform during the operation, subsequently urethane. Stomach tube inserted. Artificial respiration for 20 minutes before beginning experiment. Temperature 98.4° F.

10.50-11.10 a.m.	0.8158 g.
to 10.30	0.8346 g.
11.32	50 mgm ammonium chloride intravenously.
to 11.50	0.8842 g.
to 12.10	0.7994 g.
to 12.17	40 mgm. ammonium carbonate intravenously, one or two slight twitches. Salivary flow.
to 12.30	0.9502 g.
to 12.50	0.7890 in 20 minutes.
	This increase would be equivalent to 0.3945 in 10 minutes.
12.53	100 mgm ammonium carbonate per os.
to 1.00	0.5506 in 10 minutes.
to 1.10	0.4600 in 10 minutes.
to 1.20	0.4170 The thick saliva which has been flowing from the mouth since the carbonate was given per os now ceased.
to 1.30	0.4006
1.32	2 mgm. pilocarpine intravenously.
to 1.40	0.6954 saliva abundant.

	1.42 a.m.	Atropine was injected but as saliva was still flowing at 1.47 p.m. more was given.
to	1.50	0.4700
to	2.00	0.3926 Temperature 98.6° it has not increased above this point nor fallen below that taken at the beginning of the experiment.

Ammonium chloride and carbonate both act when given intravenously; the carbonate more strongly. The carbonate has a greater action per os than intravenously. Pilocarpine increases and atropine inhibits the flow.

Experiment XXXVII. Cat, male 2.1 K. Ether and Chloroform during operation, urethane subsequently. Artificial respiration for 20 minutes before beginning observations. Stomach tube inserted.

	1.55-2.05 p.m.	0.4401 Temperature 98.8°.
to	2.15	0.4224 at 2.13 p.m. 2 cc. wine of ipecac were given per os. no sign of vomiting but salivation.
to	2.25	0.4830
to	2.35	0.5846
to	2.45	0.4190
to	2.55	0.4256
to	3.05	0.4140
to	3.15	0.4052
to	3.21	1 cc. fluid extract of ipecac given slowly intravenously.
to	3.25	0.4460
to	3.35	0.4716
to	3.45	0.4180 Ipecac acts reflexly and also when given intravenously.

Experiment XXXVIII. Cat, female, 2.7 K. E., C. and Urethane, under artificial respiration for 20 minutes before experiment began. Temperature 37°C.

to	10.30 p.m.	0.3601 in 10 minutes
to	10.40	0.3614
to	10.50	0.3534
to	11.00	0.4432 during this time stomach tube was passed and blood pressure 110 mm. was taken.
	11.04	1.5 mgm. emetine intravenously.
to	11.10	0.3010 blood pressure fell to 80 mm. Hg., no saliva appeared.
to	11.20	0.3398 blood pressure rose again to 100 mm. temperature 36.6°C.
to	11.30	0.3160 (2 minutes lost)
	11.32	2 cc. tincture of senega per os.
to	11.42	0.4110 saliva appeared but not abundantly.
to	11.52	0.2800 temperature fell to 36.2° warmth to body
to	12.12	0.8045 in 20 minutes, 0.4027 in 10 minutes T. 36.6°
	12.17	2 cc. tincture of squill per os..
to	12.22	0.4104
to	12.32	0.3578 temperature 36.8
to	12.42	0.3636
to	12.52	0.3632 temperature 36.8 blood pressure 95
	12.52	40 mgm. ammonium carbonate intravenously
to	1.02	0.3432
to	1.12	0.3602

Result for emetine questionable. Senega per os caused an increase, centre subsequently depressed, squill per os, and ammonium carbonate intravenously ineffective.

Experiment XXXIX. Cat, female, E. C. urethane.

to 12.44 p.m.	0.3462 in 10 minutes. Temperature 38.4°C.
to 12.54	0.3390 in 10 minutes.
to 12.58	400 mgm. ammonium chloride as 20 per cent solution into stomach 12.02 saliva appeared.
to 12.04	0.3406 in 10 minutes. Temperature 28.4°C.
to 12.14	0.3506
to 12.24	0.3630 salivary flow more marked.
to 12.34	0.4494
to 12.44	0.3600 salivary flow ceased.
to 12.55	0.3408 Temperature 38.6°C.

Secretion slow but marked due to ammonium chloride per os.

Experiment XL. Cat 2.4 K. E. C. Urethane.

to 2.34 p.m.	0.3784 in 10 minutes. Temperature 38°C.
2.34	emetine 1 mgm. intravenously.
to 2.44	0.3968 in 10 minutes no saliva appeared.
to 2.54	0.4190 salivary flow. Temperature 38°C.
to 2.04	0.4162
to 2.14	0.3636.

Slight flow due to emetine intravenously.

Experiment XLI. Cat 3.9 K. E. C. urethane

to 9.40 a.m.	1.1132 in 20 minutes. 0.5566 for 10 minutes. Temperature 39°
to 9.50	0.5370 in 10 minutes.
to 10.00	0.4956 in 10 minutes.
10.00	2 cc. wine of antimony was given by the stomach tube but only part reached the stomach, as part syphoned out of the tube.
10.10	0.8207: salivary flow.
to 10.20	1.0052
to 10.30	0.8104
to 10.40	0.6495
to 10.50	0.6540
to 11.10	1.0040 in 20 minutes. = 0.5023 in 10.

Wine of antimony causes a flow of bronchial secretion.

Experiment XLII. Previously used for other purposes. Greyhound under urethane anaesthesia. Owing to length of neck good observations could be carried over a considerable length of trachea which of course preserved its normal blood supply. Careful observations were made on the movements of particles of charcoal in various parts immediately after opening. Average rate was found to be about 10 mm. per 60 seconds, but in small areas 2.4 cm. long the rate often reached 20 mm. Human saliva dropped on the membrane also was moved at the same rates. If a very thick layer was placed on it, the movement was not so fast as when it was thinner while a very thin layer rarely reached a rate of 10 mm. To samples of the

same saliva, ammonium chloride and carbonate were added in solution to produce solutions of the strength approximately of 0.007 per cent. With these solutions the rate never exceeded 20 mm. per minute. Concentrations of ammonium carbonate up to 0.05 per cent were tried without greater effect. Potassium iodide was also used but no effect was evident. Water mixed with saliva appeared as often to increase the rate to a maximum as the salt solutions. Changes in viscosity seemed of much more importance than the chemicals in solution.

In Memoriam

CHRISTIAN ARCHIBALD HERTER

Dr. C. A. Herter, Professor of Pharmacology and Therapeutics in Columbia University died of pneumonia at his home in New York City on December fifth, in the forty-sixth year of his age.

In his death the science of medicine has suffered a severe loss. An enthusiastic and tireless investigator, he made important contributions in the fields of biological chemistry, pathology, pharmacology and clinical medicine. His devotion to the higher interests of medicine was further shown in the endowment of lectureships in the Johns Hopkins University and in the University and Bellevue Hospital Medical College, in the establishment and support of the Journal of Biological Chemistry and in the maintenance of a private laboratory for chemical and medical research. He was a member of the original board of scientific directors of the Rockefeller Institute for Medical Research, appointed in 1901, and rendered valuable service to the Institute by his ripe experience in science as well as in practical medicine. At the time of his death he was treasurer to the Institute and physician to its hospital.

His sense of public duty was evidenced in his acceptance of a place on the Referee Board of the U. S. Department of Agriculture where his ability as a pharmacologist gave especial value to his counsel.

A man of attractive personality, of culture, sincerity and warmth of heart, his untimely death will be deeply mourned by those who had the privilege of his friendship.

TETANIC CONVULSIONS IN FROGS PRODUCED BY ACID FUCHSIN, AND THEIR RELATION TO THE PROBLEM OF INHIBITION IN THE CENTRAL NER- VOUS SYSTEM

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Acid fuchsin (acid magenta, Säurefuchsin, etc.) is a well known member of a large group of dyestuffs which are classed together as triphenylmethane derivatives. It is obtained by treating magenta, (rosanilin or the ordinary fuchsin of commerce) with fuming sulphuric acid, and it appears as a metallic green powder whose aqueous solutions have an intense color usually described as magenta red. Solutions of the neutral salts are colorless.

The substance finds its uses in the arts, as in the dyeing of wool and silk, and in microscopy, where it is found to be an excellent stain for connective tissue, and now and again it has been used to color wines in imitation of claret. In certain physiological experiments it is of great value. Dreser(1) was the first to use it to demonstrate to the eye the formation of acid during muscular contraction. This writer also employed the drug in his investigations (2) on the function of the kidney, while Abel (3) has made use of it as an indicator in studying the diffusion of acids in excised muscles of the frog.

Acid fuchsin has always been classed with other fuchsin as a non-toxic substance. (4) Dreser incidentally states that the frog is much more sensitive to basic fuchsin and allied compounds than to their sulpho-derivatives (as acid fuchsin) but ascribes to all of these compounds no further action than that of lowering the irritability to external stimuli and nowhere makes mention of any marked action upon the central nervous system.

Some time ago one of us (A.) made the observation while studying the diffusion of acids in muscle that acid fuchsin almost invariably causes a late tetanus in frogs quite like that produced by strychnine. This observation led to the present investigation.

Our experiments may conveniently be described under three heads:

I. The effect of acid fuchsin on the uninjured frog.

II. The effect of exercise when carried to the point of exhaustion upon frogs that have received acid fuchsin.

III. The effect of injury to or of transection of the cerebral lobes of the frog, (a) after the injection of the drug, (b) before the injection of the drug.

THE EFFECT OF ACID FUCHSIN ON THE UNINJURED FROG

The frogs used by us belonged to the species *Rana pipiens* (Gmelin) and *Rana clamata* (Daudin) (5) but fewer specimens of the latter species were available. Our experiments have been made in nearly all of the months of the year and no effects have been observed that could be ascribed to seasonal changes. We have, however, gained the impression that normal uninjured specimens of the species *clamata* are less easily thrown into convulsions by the drug than specimens of the species *pipiens*. The drug was in all cases injected into either the anterior or the posterior lymph sac, in the form of a 5 per cent solution in water, unless otherwise stated, and the dose administered varied from 1 to 8 or more mgs. pro gm. of body weight. Given in this way to normal frogs of the species named, the drug produces tetanic convulsions which in their last stages are indistinguishable from those produced by strychnine. In the first observations, made several years ago, the tetanus was first observed 20 or more hours after the injection of the drug and we were led to believe that late tetanus was the rule. But as more experiments were made it became evident that there are exceptions to this rule. In a series of forty-four frogs we have met with three that fell into convulsions in less than an hour after the administration of the drug, the intervals of time being 12, 13 and 30 minutes respectively. These three frogs be-

longed to the species *pipiens* and were small and lively specimens, the third being a spring frog (see Experiment 1). which was caught on the day preceding the injection. We cannot, however, feel certain that age is a factor in the time element and that young frogs as a rule respond with convulsions more quickly than those that are more mature. Further observations must be made in order to settle this point. A young and lively specimen of *R. clamata* which was caught in August, and which received even larger doses than those given to the three frogs cited above, did not respond with convulsions and in fact showed no abnormal symptoms during the two days in which it was under observation. We would here state that the greater number of our experiments were carried out in the spring and in the autumn, and some in the month of August, at seasons, therefore, when frogs can always be obtained in a lively condition, and we are satisfied that even the experiments which were made in the winter are in every way comparable with those made at other times.

The following protocols (Experiments 1 and 2) give the data in regard to two of the three frogs in which convulsions came on very soon after the administration of the drug:

Exp. 1. *R. pipiens*, weight 15 gms., very active, was caught March 29, 1909.

March 30, 1909, Injected 125 mgs. acid fuchsin (8.3 mgs. per gm. of body weight). Within 30 minutes extensor convulsions of the strychnine type. These continued with brief periods of relaxation for 30 minutes when respiration ceased. Exposure of the heart shows stand-still in diastole. Heart revived by mechanical stimulation and made to beat with a regular rhythm for several minutes.

Exp. 2. *R. pipiens*, weight 10 gms.

Feb. 2, 1910, 2.21 p.m. Injected 10 mgs. acid fuchsin.

2.33 p.m. Jumps about, but quickly assumes position of emprosthotonus with hind legs abducted and strongly flexed. Expiratory croak is given spontaneously.

2.37 p.m. Extensor convulsions with opisthotonus, fore limbs elevated, occasional slow abduction and adduction of legs. Duration of extensor convulsions 20 minutes when heart and respiration are found to have stopped.

In illustration of the cases most frequently observed in which the convulsions appear after the lapse of hours the following protocols are submitted:

Exp. 3. *R. pipiens*, weight 34 gms.

Oct. 22, 1909, 3.30 p.m. Injected 37 mgs. (1.1 mg. per gm. of body weight) of acid fuchsin in 10 per cent solution. Was under observation throughout the afternoon. No symptoms noted.

Oct. 23, 1909, 3.00 p.m. Was observed crawling slowly with legs dragging. When picked up tetanic convulsions of the extensor type broke out at once. Brief intervals of relaxation between the tetanic spasms, with two expiratory croaks. The convulsions continued in this way until 3.30 p.m. Examination now showed that the heart was no longer beating and that respiration had ceased.

Exp. 4. *R. pipiens*, weight 19 gms.

May 8, 1909, 2.30 p.m. Injected 50 mgs. (2.6 mgs. per gm. of body weight) of acid fuchsin in 5 per cent solution. No symptoms observed until 3.45 p.m. when restless movements made their appearance for a time.

May 10, 1909, 11.00 a.m. The animal appears to be quite normal. Now receives 50 mgs. of the drug (2.6 mgs. per gm. of body weight). No symptoms observable.

4.30 p.m. When made to crawl upon the table legs are dragged. Does not turn over when laid on back. A few moments later, grasping the animal brought on typical extensor convulsions with opisthotonus; webs of feet strongly spread. The tetanic spasms occur at the rate of two to four per minute with very brief intervals of relaxation. Respiration after a time ceased entirely, but heart could be observed to be beating well.

5.30 p.m. Legs still thrust out in extensor spasms at intervals. No further observations made.

In illustration of the difficulties encountered in producing convulsions, more especially in specimens of *R. clamata*, and also as showing what large doses may be required to bring on convulsions in freshly caught, perfectly normal frogs, the following protocols are offered:

Exp. 5. (Randolph, N. H.) Young frog, species not determined, weight about 12 gms., caught on August 10, 1910.

Aug. 12, 1910, 11.45 a.m. Injected 31 mgs. (2.6 mg. per gm. of body weight) of a 6.26 per cent solution of acid fuchsin. At 11.57 a.m. again injected 0.5 cc. of the same solution (2.6 mg. per gm. of body weight). Considerable of the solution was squeezed out, therefore it cannot be said how much was retained. The urine, however, soon became intensely red so that it is safe to assume that much of the drug was retained.

12.15 p.m. Respiratory movements slower.

1.00 p.m. Sits on rock in aquarium apparently quite normal.

3.30 p.m. No change. Is very alert. Was taken out and allowed to jump about in the grass. No indications of convulsions.

6.00 p.m. No change, is very alert.

Aug. 13 and Aug. 14. Animal is lively, no change.

Aug. 15, 9.30 a.m. Injected 1.3 cc. of a 6.26 per cent solution = 81.4 mgs. (6.8 mg. per gm. of body weight) with a syringe into various parts of the body, loss was very slight. No marked effects of any kind observable. Was watched at intervals throughout the day, no convulsions. On the morning of the 16th it was found that the frog had escaped from the out-door aquarium. We may be sure that there were no convulsions of any severity in the night as the animal would otherwise have been found dead with legs extended.

Exp. 6. (Randolph, N. H.) *R. clamata*, large, alert, male estimated to weigh from 50 to 60 gms. was caught August 10, 1910.

Aug. 12, 1910, 12.35 p.m. Injected 2.1 cc. of a 6.26 per cent solution (131.4 mgs. = 2.6 mg. per gm. of body weight) through a fine needle under the skin of various parts of the body.

1.00 p.m. No symptoms.

3.30 p.m. No change, frog sits on rock in aquarium and is very alert.

4.15 p.m. No change; no indication of convulsions when made to jump about on the ground.

6.00 p.m. No change.

Aug. 13 and Aug. 14. No change; frog is alert and shows no signs of convulsions on these days.

Aug. 15, 9.30 a.m. Injected 4 cc. of a 6.26 per cent solution (250.4 mgs. = 5 mg. per gm. of body weight) under the skin of various parts of the body; a certain amount of the fluid was squeezed out from the

dorsal lymph sac. No convulsions at any time from 9.30 a.m. to 1.55 p.m., when the anterior part of the skull was removed by a section with sharp scissors through the superior maxilla immediately posterior to and as close to the eyeballs as possible. This injury to the brain brought on convulsions in 2 minutes, as will be described in a later section. It will be seen that even large doses failed to bring on convulsions in this vigorous freshly caught frog as long as he was allowed to live with his brain intact.

That *very large quantities* of acid fuchsin may be required to produce convulsions in *R. clamata* is shown by the following protocol:

Exp. 7. (Randolph, N. H.) *R. clamata*, female, large specimen, weighing about 60 gms.

Aug. 12, 1910, 10.00 a.m. Injected 5.5 cc. of a 6.26 per cent solution (5.7 mg. per gm. of body weight) with a fine needle under the skin of various parts of the body. A small quantity was squeezed out. At 11 a.m. powerful extensor convulsions, preliminary symptoms were unfortunately missed as the animal was not kept under observation during the 10 or 15 minutes preceding the outbreak of extensor spasms. Convulsions lasted nearly an hour and feeble twitchings of the legs could still be elicited by mechanical stimulation more than an hour after the first onset of spasms.

Further instances of delayed action, that is to say, of cases in which convulsions did not appear until the brain was injured will be given in a later section. An analysis of our numerous experiments on normal frogs has shown us that tetanus will not appear, as a rule, after fairly large doses (1 to 4 mgs. per gm. of body weight of the drug) until from 1 to 20 or more hours have elapsed. In a certain number of cases no effect whatever will be produced by doses of this size or by much larger doses. This seems to hold for *R. clamata* more especially. In a number of our experiments in which no convulsions were observed during the hours of the day of the injection, the frogs were found dead on the day following

and the position of the limbs was so like that assumed ordinarily when the animals die in convulsions that it was inferred that here also the drug had not failed of its effect.

It may here be stated that repeated doses or large single doses soon cause a state of muscular weakness which is well illustrated in Experiments 3 and 4. We have here all the symptoms of a marked depression of the central nervous system. As will be shown later the drug causes at first a lengthening of the time of reflex response to stimulation of the skin. The respiratory function is also much depressed by large doses. Thus, in one experiment, a frog weighing 31 gms. received 200 mgs. of the drug. Prior to the injection the respiratory rate was 86 per minute, within 4 minutes after the injection apnoea lasting for several minutes was observed and fifty minutes later the rate was 20 per minute, the movements being shallow and irregular. Twenty-one hours later this animal was found dead.

It appears, too, from our protocols that frogs that have received only moderate doses and that have survived for hours (10 to 48) without evincing any symptoms of note, succumb rapidly to the toxic effects of the drug as soon as the convulsions appear. This is well shown, too, in later experiments (Section III) in which small doses are seen to kill the animal if its cerebral lobes are injured either before or after the injection. Inasmuch as the doses that are now fatal would not of themselves kill uninjured frogs, it would appear that the animals are poisoned not by the drug as administered, but either by its acid salt as produced in the course of the convulsions, or as a consequence of the loss of acid to the body resulting from the fixation of acid by the fuchsin or in consequence of a combination of these actions. By comparison with the prolonged action of small doses of strychnine, it is seen that the convulsions, as such, cannot be made responsible for the shortness of life after tetanus has once appeared. At the moment we are unable to throw any more light on the problems here presented.

Premonitory Symptoms. The preliminary depression of the central nervous system to which we have referred, soon disappears and leaves the animal in all respects normal unless the dose has been unduly large. The outbreak of convulsions, however, is

usually preceded by a number of premonitory symptoms of variable character. These may consist of slow crawling about, of restless movements¹ which soon give place to tetanic flexion and abduction of the legs with webs of the feet spread, and of convulsive seizures in which the animal assumes the emprosthotonic position. Flexor spasms of great intensity in which the legs are fixed in a Z-position are noted in a protocol of Section III^a, Exp. 28, as preceding the typical strychnine-like spasms. These convulsive seizures may pass for a time and the animal may then again crawl about slowly. As a rule, however, these premonitory flexor spasms continue for a short time only and then give place at once to the typical extensor convulsions. Very rarely have we seen recovery from the effects of the drug when once convulsive movements of any kind have set in. In one case, a small frog (*R. pipiens*) that had received on February 2, 1910, 1 mg. of the drug per gm. of body weight was observed to have premonitory symptoms two days later and to be seized with occasional tonic spasms in emprosthotonus. Now and again the hind legs were extended and abducted but this was the only movement that was even suggestive of the typical extensor tetanus. An hour and a quarter later the animal was quite restored and alert.

II

THE EFFECT OF PHYSICAL EXERTION WHEN CARRIED TO THE POINT OF EXHAUSTION UPON FROGS THAT HAVE RECEIVED ACID FUCHSIN

In contrast to the experiments just described, from which it is seen that acid fuchsin in average doses does not quickly cause

¹ As is well known a similar state of restlessness is seen when strychnine is administered to normal frogs, and according to Freusberg (*Arch. f. exp. Path. u. Pharmakol.*, 3, p. 376, 1875), removal of the cerebral lobes causes no change in this action of strychnine. Freusberg refers this restlessness to an increased excitability of subcortical centers. In regard to flexor spasms, we note that it is stated by Wundt (*Mechanik der Nerven*, II, p. 82, 1871), that small doses of a concentrated solution of ammonia administered subcutaneously to frogs will induce a state of increased reflex excitability, followed by reflex spasms which must be classed for the most part as *flexor convulsions*.

tetanus and in some instances fails entirely to do so, we would cite a number of experiments which illustrate the surprising effects of exercise when carried to the point of exhaustion of the volitional apparatus, in hastening the onset of tetanus in frogs that have received the drug. The exercise in all cases consisted in turning the frog on its back, catching it when it turned over on its feet and had made a spring, and repeating this manoeuvre without pause as long as the animal was able to right itself. It may perhaps be thought that when the frogs no longer righted themselves they were merely shamming or "playing dead" and were by no means exhausted. We have borne in mind the possibility that further movements might be inhibited because this protective instinct was called forth by the tactual stimulus. In many instances we have waited to see if it was a case of shamming, but the picture is very different. We have here all the appearances of exhaustion, the dragging of the legs after long periods of leaping about and the increasing slowness of every movement. We doubt also whether shamming can be maintained under the pressure of a painful stimulus. Be this as it may, the periods of time during which the frogs were "exercised" as above described, varied from five to thirty-five minutes, whereupon in the majority of cases, strychnine-like convulsions, with or without premonitory symptoms, at once made their appearance. The duration of the tetanus varied from fifteen to forty-five or more minutes, a duration that differs little if at all from that seen in "fuchsin frogs" that have not been disturbed in any way.

The amount of acid fuchsin required to bring on the convulsions under the conditions here described varies from 1.7 to 3.3 mgs. per gm. of body weight.

Premonitory symptoms of the type described in Section I may precede the outbreak of tetanus as is noted in the following protocols. These protocols do not represent all of the experiments that have been made by us in illustration of the effects of fatigue of the volitional apparatus in hastening the outbreak of the "fuchsin convulsions." A considerable number of such experiments have been made by way of demonstration and of these no record was kept. A study of the following protocols will show one in-

stance of failure to produce convulsions. Here the dose of acid fuchsin was very large but neither premonitory symptoms nor extensor convulsions were observed. In two other experiments so much of the drug had been lost by excretion that a second injection was required before exercise was effective in quickly inducing tetanus.

A study of the protocols, or of Table II, in which the results obtained are given in a more concise form, will also show that a strychnine-like tetanus, or the spasms described as premonitory symptoms, followed *immediately* upon the exercise in five out of seven cases, while in the two other cases periods of ten and twenty minutes elapsed between the cessation of the exercise and the appearance of the tetanus. We would also call attention to the periods of time that elapsed between the injection of the drug and the administration of "exercise." These periods were $\frac{1}{2}$ hour (three instances), $2\frac{1}{2}$ hours (one instance), 22 hours (one instance), 53 hours (two instances), but in these last two instances the drug was so far excreted that a second dose was required before the effects of fatigue in bringing on the convulsions could be demonstrated. It will be noted also that in none of these instances were there any indications of convulsions prior to the time of "exercise."

We conclude, therefore, that physical exertion will greatly hasten the onset of tetanic convulsions in frogs that have received acid fuchsin at any time within the preceding 24 hours. We have here a fine illustration of the close connection, existing in the frog at least, between the nutritional state of the central nervous system and its power to exercise inhibitory control over the skeletal musculature. It may be thought that the effect of exercise consists primarily in changing the neutral fuchsin of the tissues into the red or acid salt which might possibly be more of a convulsant than the neutral salt, a supposition to which allusion has already been made. We prefer, however, to assume that inhibitory power is lowered, as a result of the exercise, in those parts of the central nervous system which initiate and coördinate the movements of the skeletal muscles. No doubt the drug itself facilitates the onset of convulsions by virtue of its strychnine-like property of converting spinal and cerebral inhibitions into excitation

(Sherrington) or by virtue of an action on the cord which lessens resistance to the irradiation of impulses in all directions. We would also state here that we have seen typical strychnine-like convulsions which lasted for several minutes in a frog that received a few cc.'s of Ringer's solution in place of acid fuchsin and which had been exercised to the point of exhaustion. In both instances, that is in those in which acid fuchsin was administered as well as in the case of the frog that received Ringer's solution, the effect of the fatigue is seen to have the same end result, that of inducing convulsions. F. W. Fröhlich (6) has in recent papers called attention to the influence of fatigue, asphyxia,² lowered temperature and other influences which cause an increase in the reflex excitability of the spinal cord, and Freusberg (7) in 1875 stated that long continued electrical stimulation of one sciatic nerve in a decerebrate frog is capable of inducing tetanic convulsions which are indistinguishable from those produced by strychnine. In all of these instances in which a heightened effect is produced by ordinary stimuli in consequence of fatigue or other cause, we are dealing with the spinal cord severed from its connections with higher coördinating centers, while in our experiments these centers are retained. We have no doubt that in our experiments increased reflex excitability of the spinal cord is produced as a result of the fatigue and also perhaps as a result of the interference with respiration which is necessarily produced by the rapid and unceasing versions of the animal. But inasmuch as the entire brain is here retained, it seems rational to assume that some change has occurred also in this part of the central nervous system, and we have ventured to say inhibitory power is lessened in higher coördinating centers while reflex excitability is at the same time raised in the spinal cord.

The following protocols are given in illustration of the effects of exercise carried to the point when no further muscular response to mechanical stimulation can be elicited. The term, extensor convulsions, as found in these protocols is to be understood as the equivalent of strychnine-like convulsions.

² H. Fühner, Pflüger's Archiv. 129, p. 255, 1909 has shown that by depriving frogs of air at a temperature of 4° to 5° C. tetanoid convulsions may easily be produced.

Exp. 8. R. clamata, weight 36 gms.

- May 4, 1909, 3.25 p.m. Respirations, 72 per minute.
 3.30 p.m. Injected 100 mgs. (2.8 mgs. per gm. wt.) acid fuchsin.
 3.35 p.m. Respirations, 40 per minute.
 May 8, 1909 4.00 p.m. Injected 100 mgs. acid fuchsin.
 May 10, 1909, 11.00 a.m. Frog quite normal and alert. Injected 100 mgs. acid fuchsin. Respirations very irregular during the 20 minutes following.
 5.15 p.m. Again injected 100 mgs. of acid fuchsin.
 May 11, 1909, 3.00 p.m. *Exercise*. Frog soon tired and legs dragged, evident depression of irritability. Soon ceases to right itself when laid on his back.
 3.15 p.m. Extensor convulsions. Adductor spasms. Loud croak. Inflated abdomen becoming flaccid.
 3.25 p.m. Spasms became weaker. Relaxation between spasms.
 3.30 p.m. Stand-still of heart and respiration.

Exp. 9. R. pipiens, weight 16 gms.

- May 4, 1909, 12 noon. Injected 50 mgs. (3.1 mgs. per gm. wt.) acid fuchsin.
 2.30 p.m. Exercised for 15 minutes.
 2.45 p.m. Abdomen inflated. Respiration ceased. Incoördinate movements appear, no definite convulsions. Is returned to cage, appears to recover.
 4.00 p.m. After a very little exercise extensor convulsions appear. After a time respiration ceases. Legs jerk every $\frac{1}{2}$ to 2 minutes.
 4.40 p.m. Movements have ceased, frog dead.

Exp. 10. R. pipiens, weight 19 gms.

- May 14, 1909, 12 noon. Injected 50 mgs, (2.6 mgs. per gm. wt.) acid fuchsin.
 12.30 p.m. Exercise.
 12.35 p.m. Extensor convulsions, opisthotonus and adductor spasms. Occasionally one leg falls into tetanic spasms while the other remains unaffected.
 2.30 p.m. Heart beating feebly.

Exp. 11. R. pipiens, weight 29 gms.

- May 14, 1909, 12 noon. Injected 50 mgs. (1.7 mgs. per gm. wt.) acid fuchsin.
 12.30 p.m. Exercise.
 12.35 p.m. Frog becomes bloated. Incoördinate leg movements appear. Extensor convulsions.
 1.05 p.m. Tetanic convulsions still continue.
 2.30 p.m. Dead.

Exp. 12. R. pipiens, weight 15 gms.

- Feb. 23, 1910, 11.00 a.m. Injected 25 mgs. (1.7 mgs. per gm. wt.) acid fuchsin.
 12 noon No effect.
 Feb. 25, 1910. 2.15-2.40 p.m. Exercise.
 2.40 p.m. Fatigue. No convulsious. Injected 25 mgs. (1.7 mgs. per gm. wt.) acid fuchsin.

- 3.00 p.m. Extensor convulsions. 8 to 10 jerks per minute, abductor and adductor spasms.
 3.45 p.m. Feeble jerks.
 3.50 p.m. Heart beats, 32 per minute.
- Exp 13.* *R. pipiens*, weight 20 gms.
 Feb. 23, 1910, 11.00 a.m. Injected 25 mgs. (1.3 mgs per gm. wt.) acid fuchsin.
 12. noon No effect.
 Feb. 25, 1910, 2.15 to 2.50 p.m. Exercise.
 2.50 p.m. Fatigue. No convulsions. Injected 25 mgs. (1.3 mgs. per gm. wt.) acid fuchsin.
 3.00 p.m. Extensor convulsions. 8-10 jerks per minute.
 3.45 p.m. Feeble jerks.
 3.50 p.m. Heart rate, 30 per minute.
- Exp. 14.* *R. clamata*, weight 22 gms.
 May 14, 1909, 12 noon. Injected 50 mgs. (2.2 mgs. per gm. wt.) acid fuchsin.
 12.30 to 12.50 p.m. Exercise. No effect.
 2.30 to 2.50 p.m. Exercise.
 2.50 p.m. Marked fatigue. Incoördinate movements. Occasional extensor spasms of legs, but no typical and sustained extensor convulsions.
 4.00 p.m. Respiration very slow. Laid on back, gives an occasional convulsive shudder. Legs remain relaxed.
 4.20 p.m. Dead.
- Exp. 15.* *R. pipiens*, weight 30 gms.
 Oct. 22, 1909, 4.00 p.m. Injected 200 mgs. (6.7 mgs. per gm. wt.) acid fuchsin.
 4.30 p.m. No effect. No further observations until next day.
 Oct. 23, 1909, 11.00 a.m. Frog is alert. Exercise for 15 minutes. No effect.
 Oct. 25, 1909, 4.00 p.m. Frog is normal in every way. Exercise for 15 minutes. No effect.
 Oct. 27, 1909. }
 Nov. 1, 1909. } Frog is quite normal.

III

THE EFFECT OF INJURY TO OR PARTIAL ABLATION OF THE CEREBRAL LOBES OF THE FROG: (A) AFTER THE INJECTION OF THE DRUG; (B) BEFORE THE INJECTION.

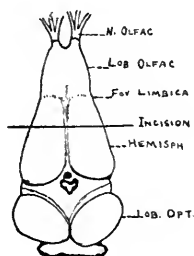
It has been shown (1) that the acid fuchsin produces tetanic convulsions in uninjured frogs in the great majority of cases: (2) that we can not induce tetanic convulsions in normal frogs with doses smaller than 1 mg. per gm. of body weight; (3) that we can not expect the convulsions to appear, as a rule, until one or more

TABLE II
The Effect of Exercise upon Frogs Which Have Received Acid Fuchsin

EXP. NO.	RANA	WEIGHT Gms	DOSE Mgm. per gm. Frog's Weight	TIME Injection to Exercise	DURATION OF EXERCISE	TIME Exercise to Symptoms	PREMONITORY SYMPTOMS		EXTENSOR TETANUS		REMARKS
							Duration		Duration		
8	Cl.	36	2.8+2.8+ 2.8+2.8	2½ hrs.	15 min.	Immed.	0	—	+	15 min.	{ Doses on 1st, 5th, and 7th days. Exercise and convulsions on 8th day. { 1½ hrs. between premonitory symptoms and tetanus
9	Pip.	16	3.1	2½ hrs.	15 min.	Immed.	+	Very Short	+	40 min.	
10	Pip.	19	2.6	½ hr.	5 min.	Immed.	—	—	+	not noted	
11	Pip.	29	1.7	½ hr.	5 min.	Immed.	+	Very Short	+	35+ min.	
12	Pip.	15	1.7+1.7	53 hrs.	25 min.	20 min.	0	—	+	45+ min.	{ 2nd dose immediately after exercise. 2nd dose immediately after exercise. { 1 hr. and 40 min. between 2 exercises. Convulsions not typical. { 29 hrs. between 2 exercises. { No effect.
13	Pip.	20	1.3+1.3	53 hrs.	35 min.	10 min.	0	—	+	45+ min.	
14	Cl.	22	2.2	½ hr.	20 +20 min.	Immed.	+	Very Short	+	?	
15	Pip.	30	6.7	19 hrs.	15 +15 min.	—	0	—	0	—	

hours have elapsed; (4) that exercise carried to the point when no further response is obtainable with mechanical stimuli lessens inhibitory control and hastens the outbreak of convulsions.

But more impressive is the effect of partial ablation of the cerebral lobes, or of injury to their substance along a transverse line which lies at one-third of the distance from the fovea limbica (or posterior edge of the olfactory lobes) and the occipital poles of the cerebral lobes as shown in the accompanying figure. In frogs that have received acid fuchsin and whose cerebral lobes were then transected as here described, we find (1) *that general extensor convulsions appear with certainty*; (2) *that these occur within a few minutes after the operation*, and (3) *that they take place after doses as small as 0.35 mg. per gm. of body weight*. The amount of



BRAIN of FROG (dorsal view) After Ecker.

drug actually distributed in the system at this time must be greatly less than that which was injected. The experiment is one of the most striking that can be made with a convulsant drug.

That haemorrhage or surgical interference (irrespective of the part injured) can not be made responsible for the above effect is shown by the fact that removal of the top of the skull, injury to or removal of the olfactory lobes, ablation of the most anterior portion of the cerebral lobes by section close to the *fovea limbica*, or extirpation of the eyeballs, are one and all without effect in hastening the onset of convulsions. Transection of the cord just below the medulla or complete removal of the entire brain by decapitation or destruction of the entire brain and medulla by pithing, has the same effect as partial ablation of the cerebral lobes only.

In our experiments upon the cerebral lobes we have proceeded in various ways. In many experiments we first removed the top of the skull and laid bare the cerebral lobes. Partial removal of the lobes or injury of their substance at a definite point is then only a matter of using the proper instruments. In other cases we have transected the upper maxilla with a pair of sharp scissors, making the section just back of the eyes and pushing the eyeballs as far forward as possible while making the cut. It was found when decapitation was performed in this way that less than the anterior half of each cerebral lobe was removed. We have found this to be the quickest and easiest way of demonstrating the effects of partial ablation of the brain. In a number of experiments the brain after operation was hardened in alcohol and subsequently examined, and in this way we have convinced ourselves that it is only necessary to remove the anterior third of the cerebral lobes in order to bring on convulsions quickly in an acid fuchsin frog.

The following protocols (Experiments 16 to 28, inclusive) are offered in illustration of these statements above. It will be seen (1) that the drug was given in doses varying from 0.35 mg. to 2.3 mgs. per gm. of body weight; (2) that the time intervening between the injection of the drug and the operation on the brain varied from 8 minutes to $4\frac{1}{2}$ hours; (3) *that after the operation on the brain it requires only from $\frac{1}{2}$ to 13 minutes for the effects of the drug to become apparent, and that violent exercise just prior to the operation causes convulsions to come on immediately*; (4) that the period of shock after the operation is very brief, and that the premonitory symptoms in this series of experiments, as a rule, occupy but a few minutes; (5) that the duration of the tetanus has been from ten minutes to seventeen hours.

Attention is also called to Experiment 25, in which three frogs (A, B and C) were used with the following results:

Frog A. A dose of 0.28 mg. per gm of body weight (a quantity too small to induce convulsions) produced a marked lengthening in the time of response to stimulation of the skin with acid. This depression remained unchanged for fifty minutes, when transverse section of the cerebral lobes was performed, which operation caused no decided change in irritability and did not cause convulsions.

Frog B. A dose of 0.53 mg. per gm. of body weight, a quantity which will induce convulsions only in frogs with brain injury, produced a state of depression which in less than half an hour was followed by a marked increase in irritability to stimuli applied to the skin. Transection of the cerebral lobes was followed by tetanic convulsions in ten minutes.

Frog C. This frog received 0.2 cc. of Ringer's solution in place of acid fuchsin and served as a control to Frogs A and B, being suspended from the lower jaw in the same manner as A and B. In the fifty minutes that preceded the transection of the cerebral lobes no changes in irritability could be detected. This operation, however, caused a definite extensor tetanus differing only from that produced by strychnine or acid fuchsin in the shortness of its duration and in the absence of all after-effects. Further experimentation taught us that this brief tetanus with Ringer's solution could be obtained only in frogs that were suspended from the lower jaw for two hours before the cerebral lobes were transected. Various factors evidently play a rôle in the production of the tetanus in this experiment. We have here to consider the combined effects of the injury to the cerebral lobes of the chemical alterations that follow upon the prolonged struggling to get free, of the alteration in the blood supply in consequence of the suspension, and of partial asphyxia in consequence of the interference with respiration. The absence of all after effects differentiate this case from those described under A and B.

Experiments illustrating III (a)

Exp. 16. R. pipiens, weight 43 gms.

Nov. 10, 1909, 11.40 a.m. Injected 100 mgs. (2.3 mgs. per gm. wt.) of acid fuchsin.

4.00 p.m. Found slowly crawling about. When placed on floor gave two long leaps landing in a sprawled out position.

4.05 p.m. Destroyed medulla and brain by pithing. Suspended.

4.08 p.m. Increased irritability to tactile stimuli. Slow rhythmic flexions and relaxations of legs alternately. Spreads webs of toes. Then con-

vulsions, tetanic extension of legs with opisthotonus. Relaxation between attacks. Attacks induced at will by tactile stimuli. After a time cut cord at level of fifth vertebra and obtained convulsions of legs for several minutes after.

Exp. 17. R. pipiens, weight 12 gms.

- Jan. 12, 1910, 4.27 p.m. Injected 25 mgs. (2.1 mgs. per gm. wt.) acid fuchsin
 4.39 p.m. Some depression. Transverse section through medulla.
 4.41 p.m. Stimulation of skin with weak acid causes flexion of legs followed by incoördinate movements of extension, abduction and adduction.
 4.49 p.m. Violent spontaneous movements. Rapid flexions and relaxations. Then semiflexed position of arms and legs assumed.
 4.52 p.m. Spontaneous convulsions. Opisthotonus and hyperextension of legs.
 5.00 p.m. Incoördinate movements again followed by tetanus. Typical "strychnine position" assumed, back arched, arms flexed with hands interlocked, legs fully extended.
 5.30 p.m. Extensor tetanus continues.

Exp. 18 R. pipiens, weight 19 gms.

- Feb. 21, 1910, 2.35 p.m. Injected 10 mgs. (0.5 mg. per gm. wt.) acid fuchsin and put in ice box. Irritability depressed.
 4.15 p.m. Removed from ice. Transverse section through middle of cerebral lobes.³ Suspended.
 4.20 p.m. Rapid flexions and relaxations of legs alternately.
 4.25 p.m. The same alternating with periods of extensor tetanus in which arms are held abducted and extended and curved outward.
 4.30 p.m. Tetanus. Arms brought around in front and digits interlaced. Legs brought together and strongly extended.
 4.35 p.m. Tetanus still continues.

Exp. 19. R. pipiens, weight 19 gms.

- Feb. 21, 1910, 3.52 p.m. Injected 25 mg. (1.3 mg. per gm. wt.) acid fuchsin.
 4.06 to 4.19 p.m. Kept exercising in this interval.
 4.19 p.m. Transverse section through middle of cerebral lobes. Suspended. Arms at once held abducted and extended, legs abducted, extended and curved outward.

³ Here and in all later protocols in which this statement occurs the olfactory lobe is included as a part of the cerebral lobe. A section made in this way removes about one-third of the cerebral lobe proper.

- 4.22 p.m. Movements of abduction and extension of legs.
 4.23 p.m. Opisthotonus, tetanus.
 4.24 p.m. Arms brought around in front of thorax. Spontaneous jerks between longer spasms.
 4.35 p.m. Tetanus continues.
- Exp. 20.* R. pipiens, weight 15 gms.
 Feb. 8, 1910, 3.13 p.m. Injected 13 mgs. (0.9 mg. per gm. wt.) acid fuchsin.
 4.12 p.m. No effect. Exposed right sciatic nerve; stimulated this nerve with strong induction shocks. Tetanus in right leg, but no other effect. Cut right sciatic nerve.
 4.21 p.m. Transverse section through middle of cerebral lobes.
 4.26 p.m. No effect. Stimulated central end of cut sciatic with induction shocks. Extensor convulsions produced in ten seconds. (Right leg flaccid.)
 4.28 p.m. Spontaneous extensor tetanus.
- Exp. 21.* R. pipiens, weight 30 gms.
 Feb. 9, 1910, 3.53 p.m. Removed skull over cerebrum.
 4.00 p.m. Injected 15 mgs. (0.5 mg. per gm. wt.) acid fuchsin.
 4.17 p.m. Frog is alert and normal. Beginning with olfactory lobes injured both sides of brain with a needle five or six places anterior to middle of cerebrum. No symptoms.
 4.22 p.m. Inserted needle first into one cerebral lobe, then into the other near the mid-level of the lobes. Frog relaxes.
 4.24 p.m. Extensor tetanus, opisthotonus and adductor spasms. Relaxes between jerks.
 4.28 p.m. Croak. Digits of hands interlocked.
 4.39 p.m. About twice a minute the following attack takes place. Frog lies on belly, relaxed, becomes uneasy, rises on all fours, plunges violently forward in a tetanic spasm. Then relaxes again and again plunges forward in a spasm. Respiration 40.
 4.47 p.m. Tetanus.
- Feb. 10, 1910, 11.00 a.m. Tetanus still continues.
- Exp. 22.* R. pipiens, weight 10 gms.
 Jan. 26, 1910, 4.38 p.m. Injected 12.5 mgs. (1.25 mg. per gm. wt.) acid fuchsin.
 4.49 p.m. Transverse section through middle of cerebral lobes.
 4.50½ p.m. Spontaneous extensor convulsions, relaxes between spasms.
 5.00 p.m. Heart beating well. Tetanus continues.
 5.20 p.m. Condition as before. Tetanus still continues.
- Exp. 23.* R. pipiens, weight 10 gms.
 Jan. 26, 1910, 4.44 p.m. Injected 12.5 mgs. (1.25 mg. per gm. wt.) acid fuchsin.

- 5.07 p.m. No effect. Transverse section through olfactory lobes.
- 5.13 $\frac{1}{4}$ p.m. No effect. Transverse section farther back, approximately through middle of cerebral lobes.
- 5.13 $\frac{1}{2}$ p.m. Extensor convulsions. Relaxes between spasms.
- 5.19 p.m. Transverse section through medulla. Relaxation.
- 5.20 $\frac{1}{2}$ p.m. Tetanus again.
- Exp. 24.* R. pipiens, weight 10 gms.
- Feb. 2, 1910, 2.45 p.m. Injected 10 mg. acid fuchsin.
- 3.44 p.m. Enucleated left eye.
- 3.54 p.m. No effect. Enucleated right eye.
- 4.20 p.m. No effect. Transverse section through middle of hemispheres.
- 4.25 p.m. Typical extensor tetanus.
- Exp. 24 A.* R. pipiens, weight 35 gms.
- Feb. 2, 1910, 3.00 p.m. Skull removed over cerebrum.
- 3.50 p.m. Injected 25 mgs. acid fuchsin (0.8 mg. per gm. wt.)
- 4.07 p.m. Removed right olfactory lobe.
- 4.12 p.m. No effect. Removed left olfactory lobe.
- 4.17 p.m. No effect. Transverse section through middle of cerebral lobes. Depression.
- 4.30 p.m. Convulsions. Opisthotonus, followed by incoördinated sprawling movements and flexion and relaxation of legs alternately.
- 4.50 p.m. Extensor convulsions as after strychnine.
- 5.15 p.m. These continue.
- Exp. 26.* (Randolph, N. H.) R. clamata, female, weight about 50 gms., was caught a few days before.
- Aug. 24, 1910, 10.51 $\frac{1}{2}$ a.m. Injected 82.4 mgs. acid fuchsin (about 1.6 mg. per gm. weight.) No symptoms.
- 11.07 $\frac{1}{2}$ a.m. Section of upper maxilla, just back of eyeballs, these being pushed forward before completing the section.
- 11.08 $\frac{1}{4}$ a.m. Convulsions begin. Are of flexor type at first. Soon have typical extensor tetanus.
- 11.45 a.m. Can still induce fine extensor tetanus by touching skin.
- 12.30 p.m. Can no longer induce convulsions.

The unremoved portion of the brain of this frog was hardened in alcohol and an examination of it later showed that we had removed what we judged to be the anterior third of each cerebral lobe.

Experiment 25

Feb. 4, '10	A R. piplens Wt. 18 Gm.		B R. piplens Wt. 19 Gm.		C R. piplens Wt. 17 Gm.	
P.M. 2.15	SUSPENDED		SUSPENDED		SUSPENDED	
	Respiration	Time of Response to 1 per cent HCl Applied to Thigh	Respiration	Time of Response to 1 per cent HCl Applied to Thigh	Respiration	Time of Response to 1 per cent HCl Applied to Thigh
4.15	26	—	36	1 sec.	32	—
4.20	32	1 sec.	32	8 sec.	26	4 sec.
4.30	Injected	{ 5 mg. (0.3 mg. per gm. wt.) acid fuchsin }	Injected	{ 10 mg. (0.5 mg. per gm. wt.) acid fuchsin }	Injected	0.2 cc. Ringer's Sol.
4.35	0	No response	28	No response	16	1 sec.
4.40	20	33 sec.	38	21 sec.	—	4½ sec.
4.45	2	No response	21	8 sec.	32	—
4.50	12	36 sec.	32	7½ sec.	40	5½ sec.
4.55		28 sec.		7 sec.		3½ sec.
5.00	28	9 sec.	24	½ sec.	20	—
5.05		No response		1 sec.		5½ sec.
5.10		21 sec.		1 sec.		5½ sec.
5.15	26		44	½ sec.	0	5 sec.
5.20	Transverse section through middle of cerebral lobes in all three cases.					
	REFLEX TIME		REFLEX TIME		REFLEX TIME	
	5.22½ p.m.	38½ sec.	5.22½ p.m.	13 sec.	5.20 p.m.	Immediate extensor tetanus lasting only 9 minutes. Legs first, then arms (after incoördinate movements of arms).
	5.31 p.m.	25 sec.	5.30 p.m.	Spontaneous incoördinate movements.		
	5.40 p.m.	Feeble incoördinate movements in response to touch.	5.34 p.m.	Extensor tetanus alternating with incoördinate movements, alternate flexions of legs.	5.29 p.m.	Relaxed. Croak. No recurrence of tetanus.
6 p.m.	No convulsions.		5.37 p.m.	Extensor tetanus		Respiration regular. Sits up like normal frog.
Feb. 5, '10						
10.30 a.m.	Responds to tactile stimulus by slow flexions and relaxations of arms and legs. Resp. 1 or 2 per min.		Extensor tetanus continues. Feeble jerks in response to tactile stimulus.			Found sitting up like normal frog.
11 a.m.	Cold bath		Resp. 1 or 2 per min. when handled.			
11.05 a.m.	No response to 5% HCl		Cold bath			Cold bath
11.20 a.m.	No response to 5% HCl		No response to 5% HCl			No response to 5% HCl
			No response to 5% HCl			Responds to 5% HCl after 26 sec.

Exp. 27. *R. clamata*. For details previous to the operation on the brain see Experiment 6 of Section I, where this case was used to illustrate the difficulty of inducing convulsions in some instances, especially in the species *clamata*.

Aug. 15, 1910, 1.55 p.m. Transection of upper maxilla just back of eyeballs, as in Experiment 26. Frog allowed to jump twice.

1.57 p.m. Immediately falls into convulsions which are like those produced by strychnine.

2.07 p.m. The convulsions have given place to relaxation.

2.37 p.m. Touching skin induces only feeble jerks of the legs, but these are distinctly convulsive in character.

Exp. 28. (Randolph, N. H.) *R. clamata*, large male, weight about 59 gms.

Aug. 15, 1910, 3.19 p.m. Injected 110 mgs. (2.2 mg. per gm. wt.) of acid fuchsin in various places (6.26 per cent sol.). Some lost. No signs of convulsions.

3.28 p.m. Removed anterior portion of brain by transection of the upper maxilla just back of the eyeballs and as close as possible to these.

3.29 p.m. Flexor and adductor spasms of the most violent type. Legs firmly fixed in tetanus in a Z-shape for a time.

3.30 p.m. Extensor spasms appear. Typical tetanus as after strychnine.

3.40 p.m. Entire relaxation. Irritation of skin induces feeble spasms. Brain is dissected.

III (b)

Protocols of experiments illustrating the effect of acid fuchsin injected *after* injury to the brain. In two of these experiments the anterior third of the cerebral lobes was removed two and seven days respectively before the acid fuchsin was administered.

Exp. 29 *R. pipiens*, weight 19 gms.

May 6, 1909. Destroyed medulla and brain by pithing. Within one-half hour injected 150 mg. (7.9 mg. per gm. wt.) acid fuchsin. Within 5 minutes, extensor tetanus.

Exp. 30. *R. pipiens*, weight 14 gms.

Nov. 4, 1909, 4.45 p.m. Destroyed medulla and brain by pithing.

5.05 p.m. Injected 37 mg. (2.6 mg. per gm. wt.) acid fuchsin.

5.08 p.m. Extensor tetanus in response to weak acid stimulus.

Exp. 31. *R. pipiens*, weight 17 gms.

Nov. 4, 1909, 5.00 p.m. Destroyed medulla and brain by pithing.

5.07 p.m. Injected 25 mg. (1.5 mg. per gm. wt.) acid fuchsin.

5.11 p.m. Violent incoördinate movements followed by extensor convulsions.

TABLE III (a)
The Effect of Injury to Cerebrum of Frogs Injected Previously with Acid Fuchsin

EXP. NO.	RANA	WEIGHT GR.	DOSE M'g mls per gm. Frog's Weight	TIME Injection to Injury	POINT OF INJURY	TIME Injury to Symptoms	PREMONITORY SYMPTOMS		EXTENSOR TETANUS		REMARKS
							Duration		Duration		
16	Pip.	43	2.3	4½ hrs.	Medulla	3 min.	+		+	Few min.	
17	Pip.	12	2.1	12 min.	Medulla	2 min.	+		+	40 min.	
18	Pip.	19	.5	40 min.	Ant'r ¼ Cerebrum	5 min.	+		+	10+ min.	
19	Pip.	19	1.3	27 min.	Ant'r ¼ Cerebrum	Immed.	+		+	16+ min.	Exercise 13 min. before injury
20	Pip.	15	0.9	8 min.	Ant'r ¼ Cerebrum	5 min.	+		+	not noted	
21	Pip.	30	0.5	22 min.	Ant'r ¼ Cerebrum	7 min.	0		+	17 hrs.	Injury with needle point
22	Pip.	10	1.3	11 min.	Ant'r ¼ Cerebrum	1½ min.	0		+	½ hr.	
23	Pip.	10	1.3	29 min.	Ant'r ¼ Cerebrum	1½ min.	0		+	7+ min.	
24	Pip.	10	1.0	95 min.	Ant'r ¼ Cerebrum	5 min.	0		+	not noted	
24A	Pip.	30	0.8	27 min.	Ant'r ¼ Cerebrum	13 min.	+		+	25 min.	
25A	Pip.	18	0.3	51 min.	Ant'r ¼ Cerebrum	20 min.	0		0	—	{Subminimal dose. Suspended 2 hrs. before injection
25B	Pip.	19	0.5	51 min.	Ant'r ¼ Cerebrum	10 min.	+		+	17 hrs.	Suspended 2 hrs. before injection
26	Cl.	50	1.6	16 min.	Ant'r ¼ Cerebrum	¾ min.	+		+	30+ min.	
27	Cl.	50	2.6+5.0	4 hrs. & 25 min.	Ant'r ¼ Cerebrum	2 min.	0		+	40 min.	= Exp. 6
28	Cl.	50	2.2	9 min.	Ant'r ¼ Cerebrum	1 min.	0		+	11+ min.	

Exp. 32. *R. pipiens*, weight 17 gms.

- Feb. 7, 1910, 2.35 p.m. Transverse section through middle of hemispheres.
 2.54 p.m. Injected 10 mg. (0.6 mg. per gm. wt.) acid fuchsin.
 3.01 p.m. Jumping.
 3.01½ p.m. Extensor convulsions, incoördinate and jumping movements in intervals.
 3.04 p.m. Croak. Extensor convulsions. Relaxation between spasms.
 3.08 p.m. Ten jerks per minute.
 4.00 p.m. Still feeble jerks.
 4.30 p.m. Heart beating 18 per minute.

Exp. 33. *R. pipiens*, weight 19 gms.

- May 6, 1909, 12 noon. Destroyed medulla and brain by pithing. Suspended. Reflex response to 1 per cent acetic acid, 7 seconds.
 12.30 p.m. Injected 100 mgs. (5.3 mgs. per gm. wt.) acid fuchsin.
 12.40 p.m. Injected 50 mgs. (2.6 mgs. per gm. wt.) acid fuchsin.
 12.45 p.m. Reflex response to 1 per cent acetic acid, 1 second.
 12.58 p.m. Incoördinate convulsions followed at once by extensor tetanus.

Exp. 34. *R. clamata*, weight 17 gms.

- Nov. 5, 1909, 4.00 p.m. Destroyed medulla and brain by pithing. Suspended.
 4.08 p.m. Injected 6 mgs. (0.35 mg. per gm. wt.) acid fuchsin.
 4.13 p.m. Spontaneous rapid flexions and relaxations of legs. Great irritability to acid.
 4.25 p.m. Injected 25 mgs. (1.5 mg. per gm. wt.) acid fuchsin.
 4.29 p.m. Acid stimulus causes rapid flexions and relaxations.
 4.33 p.m. Acid stimulus causes rapid flexions and relaxations, followed by hyperextension of legs which become knotted behind body. Opisthotonus.
 4.40 p.m. Incoördinate movements followed by extensor tetanus.

Exp. 35. *R. pipiens*, weight, 27 gms.

- Nov. 5, 1909, 3.43 p.m. Destroyed medulla and brain by pithing. Suspended.
 4.08 p.m. Injected 12.5 mgs. (0.5 mg. per gm. wt.) acid fuchsin.
 4.12 p.m. Rhythmical flexions and relaxations of legs, spreading of webs of toes. Violent extensor jerks. Legs hyperextended and abducted high above head. Opisthotonus.
 4.19 p.m. Rhythmical flexions and relaxations of legs. Great irritability to tactile and acid stimuli.
 4.25 p.m. Injected 2.5 mgs. (0.9 mg. per gm. wt.) acid fuchsin. Responds to acetic acid stimulus by rapid flexions.
 4.30 p.m. Spontaneous extensor convulsions. Spasms with curving of legs, one outward, one inward.

Spasms with curving of legs, both outward. Strychnine position assumed (straight extension).

- 4.33 p.m. Tetanic jerk brought on by acid stimulus.
 4.35 p.m. Tetanic jerk brought on by tactile stimulus.
 4.38 p.m. Tetanic jerk brought on by tactile stimulus. Refractory period (1 or 2 minutes) when jerks can not be brought on.
 5.16 p.m. Extensor tetanus continues.
- Exp. 36.* R. pipiens, weight 21 gms.
 Jan. 7, 1910, 3.10 p.m. Destroyed medulla and brain by pithing. Suspended.
 3.49 p.m. Reflex response to weak acid, 8-10 seconds.
 4.02½ p.m. Injected 50 mgs. (2.4 mg. per gm. wt.) acid fuchsin.
 4.07 p.m. Violent incoördinate movements. Legs thrown high, arms extended. Rapid jerks of legs. Relaxation in about ¾ minute.
 4.08 p.m. Very rapid flexions and relaxations of legs, legs pushed violently against nearby objects.
 4.13 p.m. Extensor convulsions, extreme opisthotonus, arms extended and crossed. Legs in extension but slightly abducted. Webs of toes spread.
 4.16 p.m. Several spontaneous jerks within ¼ minute. Jerks become less frequent and more feeble.
 4.24 p.m. Tetanic jerk in response to weak acid.
 4.29 p.m. No response to tactile stimulus.
 4.32 p.m. Extensor response to tactile stimulus (tetanic jerk).
 4.35 p.m. Extensor response to tactile stimulus.
- Exp. 37.* R. pipiens, weight 12 gms.
 Feb. 9, 1910, 1.35 p.m. Suspended.
 3.39 p.m. Transverse section through middle of hemispheres.
 4.05 p.m. No result. Climbs, etc., as before cutting.
 Feb. 11, 1910. 3.10 p.m. Lively.
 3.15 p.m. Reflex time, weak acid stimulus, 1 second.
 3.20 p.m. Injected 10 mg. (0.8 mg. per gm. wt.) acid fuchsin.
 3.21 p.m. Reflex time, 22½ seconds.
 3.25 p.m. Extensor convulsions.
 3.25-3.50 p.m. 2 to 3 jerks per minute.
- Exp. 38* (Randolph, N. H.) R. clamata, large male, probable weight about 60 gms., was caught a few days before.
 Aug. 15, 1910, 10.00 p.m. Removed anterior part of the upper maxilla by transection just back of the eyeballs. Part of left eye remained attached to trunk. As explained, this operation removes only about ½ of the cerebrum. Frog placed in aquarium. No shock, frog sits up like an uninjured frog.

- Aug. 17, 1910, 10.00 a.m. Frog is in fine condition. Injected 2.6 cc. of a 6.26 per cent solution (2.7 mg. per gm. wt.) of acid fuchsin under the skin in various parts of the body. After withdrawing the needle the frog was allowed to make two leaps, and within one-half minute when the animal was seized with the hand and one minute after the injection was made a typical tetanus, as after strychnine, made its appearance.
- 10.12 p.m. Lies flat and motionless, a feeble tetanus of short duration can be elicited by strongly stimulating the skin.
- 2.12 p.m. Feeble spasms of legs can still be elicited. Brain removed and placed in alcohol.
- Exp. 39.* (Randolph, N. H.) R. clamata, large specimen, male, recently caught. Weight, probably 60 gms.
- Aug. 17, 1910, 12 noon. Removed skull over cerebral lobes. Took out what was judged to be the anterior third of each cerebral lobe. No shock. Frog appears quite normal. Is placed in an aquarium until August 24, when wound looks well. Frog is alert and in good condition.
- Aug. 24, 1910, 10.20½ a.m. Completed the injection of 80 mgs. (1.3 mg. per gm. wt.) of acid fuchsin (6.26 per cent sol.). Considerable of the solution escapes from the point of injection. Is placed on the ground and made to jump a few times.
- 10.29 a.m. Depression has set in. Does not jump when touched.
- 10.30 a.m. Flexor spasms when tried to jump. A touch now evoked typical extensor convulsions as seen after strychnine, which continued until 10.45 a.m.
- 10.45 a.m. Tetanus of brief duration when frog is touched.
- 10.58 a.m. Convulsions, or spasms, can no longer be elicited. Brain is placed in alcohol for study.

That the above results are due to a specific action of acid fuchsin itself and that they can not be ascribed to other substances, has been shown by control experiments. Thus, boiling the drug with alkalies has failed to show the presence of salts of ammonia. It has also been shown that neither mineral nor organic acids will induce convulsions and to prove further that the acid properties of our drug are not as such responsible for the convulsions, we have injected a solution which was made neutral to litmus. This

TABLE III (b)
The Effect of Injury to Cerebrum of Frogs Before Injection of Acid Fuchsin

EXP. NO.	RANA	DOSE Mgms. Per gm. Frog's Weight	POINT OF INJURY	TIME Injury to Injection	TIME Injection to Symptoms	PREMONITORY SYMPTOMS		EXTENSOR TETANUS		REMARKS
						Duration		Duration		
29	Pip.	7.9	Medulla	$\frac{1}{2}$ hr.	5 min.	0	—	+	not noted	{ First dose ineffective. Second dose 10 min. later.
30	Pip.	2.6	Medulla	20 min.	3 min.	0	—	+	not noted	
31	Pip.	1.5	Medulla	7 min.	4 min.	+	very short	+	not noted	
32	Pip.	0.6	Ant'r $\frac{1}{3}$ Cerebrum	19 min.	7 min.	+	$\frac{1}{2}$ min.	+	1 hr.	
33	Pip.	5.3+2.6	Medulla	30 min.	18 min.	+	very short	+	not noted	{ First dose gave clonic symptoms. Second dose 17 min. later. 2nd dose given during premonitory symptoms, 5 min. before tetanus.
34	Cl	0.4+1.5	Medulla	8 min.	4 min.	+	4 min.	+	not noted	
35	Pip.	0.5+0.9	Medulla	25 min.	4 min.	+	18 min.	+	46+ min.	
36	Pip.	2.4	Medulla	52 $\frac{1}{2}$ min.	4 $\frac{1}{2}$ min.	+	6 min.	+	22+ min.	
37	Pip.	0.8	Ant'r $\frac{1}{3}$ Cerebrum	2 days	5 min.	0	—	+	25+ min.	{ First dose ineffective. Second dose 10 min. later.
38	Cl.	2.7	Ant'r $\frac{1}{3}$ Cerebrum	2 days	$\frac{1}{2}$ min.	0	—	+	2 hrs. & 12 min.	
39	Cl.	1.3	Ant'r $\frac{1}{3}$ Cerebrum	7 days	8 $\frac{1}{2}$ min.	+	1 min.	+	15 min.	

solution was injected into a frog whose brain and medulla had been destroyed, with the result that extensor convulsions of more than half an hour's duration were produced.

Specimens of acid fuchsin obtained from two manufacturers were used in our experiments without difference in results.

We would also state that frogs are the only cold-blooded animals on which we have thus far made experiments with this compound and that in a few experiments with it on warm-blooded animals (dogs and rabbits) we failed to note a convulsant action.

DISCUSSION

Acid fuchsin behaves in many ways like strychnine though it is less powerful and less rapid in its action. The flexor spasms and flexor convulsions induced by it are, however, more strongly developed than those produced by strychnine. Langendorff (8) has shown that flexor convulsions may occur in frogs that are slowly poisoned with strychnine and that flexor spasms of the legs may be observed when a minute crystal of strychnine is placed on the distal surface of the transected medulla.

Our drug also differs markedly from strychnine and other convulsants hitherto examined in that its action is so very greatly accelerated by muscular exertion and by removal of the anterior third of the cerebral lobes. Even when the injury to the brain (removal of the anterior third) antedates the administration of the drug by a week this effect remains unaltered. Further experiments must determine whether a frog thus operated upon will remain permanently hypersensitive to the drug.

A frog with its cerebral lobes intact is not affected in the least by a quantity of the drug which suffices to throw the other into convulsions in a few minutes. It is evident that in a frog whose cerebral lobes have been transected the ratio of reciprocal inhibitory and exciting influences is altered, though no outward evidence is given that this ratio is disturbed. We have here an instance of drug action which bears upon the most intricate problems of the central nervous system.

Our drug must also be compared in its action with ammonium

chloride whose action on the central nervous system has been submitted to a critical analysis by Yourinsky (9). In testing the reflexes of a "spinal" frog, this author found that medium doses of this drug cause a marked increase in reflex excitability lasting from half an hour to an hour and a half. When, however, the same tests were made two or three days after extirpation of the cerebral lobes a period of depression appeared which preceded the period of increased reflex excitability. The period of augmentation of the reflexes was now limited to ten or twenty-five minutes and was followed by convulsions as before. A series of tests was then made in order to learn whether the cerebral lobes played a part in this depression of reflex excitability, and to this end the behavior of normal frogs was compared with that of frogs whose cerebral lobes had been removed. No difference was observable, ammonium chloride induced in both series of frogs a depression of reflex excitability which was followed as before by a period of augmented excitability. When the brain section was made just back of the optic lobes the same order in the stages of reflex activity was noted. Only when the spinal cord alone was retained was the stage of depression of reflex activity absent.

Yourinsky refers this depression of reflex activity to inhibitory influences which emanate from the mid-brain and the medulla oblongata and act upon the reflex mechanisms of the cord. As the action of the drug progresses, this depression finally passes into excitation as a consequence either of the growing irritability of the spinal cord itself, or of a weakening of the inhibitory influences which emanate from the higher centers. In frogs in which the spinal cord alone is retained, the stimulating action of the drug upon this part of the central nervous system, is not diminished or altered by impulses that proceed from higher centers and hence manifests itself at once in a marked rise in reflex excitability.

Yourinsky next proceeds to analyze the more complicated nervous phenomena that are seen when normal as well as decerebrate frogs are poisoned with ammonium chloride. And here the main question is a consideration of "the general depression of the nervous system" which is observed soon after (7 to 17 minutes) the administration of the drug. In normal frogs the drug

induces so complete a prostration in the course of half an hour that the strongest stimuli can not evoke more than feeble muscular twitches. If the animal is now placed on its back it is unable to right itself. This stage of depression may last from 15 to 60 minutes when it is followed by a period of excitability which soon passes into general tetanus.

In frogs whose cerebral lobes were removed some days previously, the period of depression was hardly noticeable; but "the second period, that of increased excitability of the nervous system as shown in a heightened reflex excitability and in convulsions, if not more accentuated, was at least as well represented as in normal frogs, though appearing more quickly than in these." That the convulsions appear earlier in frogs that have been deprived of their cerebral lobes is hardly borne out by a study of our author's protocols. In two experiments with normal frogs the times that elapsed before the appearance of general tetanus were 25 and 61 minutes, respectively, while in the case of three decerebrate frogs the periods were 27, 44 and 29 minutes respectively. The dose per gram of body weight is not stated and it must therefore remain doubtful whether removal of the cerebral lobes has really hastened the outbreak of convulsions. There can be no doubt, however, that decerebrate frogs fail to exhibit the great nervous and muscular prostration which is induced by ammonium chloride in normal frogs. Yourinsky concludes that "the phenomena of depression⁴ which are seen in frogs poisoned with ammonia have as their cause a controlling action of the cerebral lobes upon the mid brain in the same manner as this part of the brain controls the reflex excitability of the spinal cord."

Goltz (10) long ago made an assumption of this sort to explain the increased reflex excitability of the decerebrate frog. His words are as follows: "The brain is in constant activity during life and accordingly excitations are continually passing down from it to

⁴Loeb holds that "it is possible that certain impulses flow constantly from the cephalic to the lower parts of the central nervous system," and that "the interruption of these influences may be responsible for the condition which we call shock-effects and which may be transitory." *Physiology of the Brain*. 1900. pp. 146 and 126.

the centers of the spinal cord. Even if these impulses are not always of sufficient strength to induce muscular contractions, they are nevertheless in a position to weaken the reflex power of the spinal centers. After decapitation this disturbing influence of the brain ceases and the reflex excitability of the spinal centers now appears to be increased."

Since these words were written many experiments have been made which have given greater definiteness to our ideas in regard to the play of forces in the central nervous system (11), but we can here refer only to a few experiments that deal with the action of poisons upon the coördinating mechanisms of the central nervous system. Sherrington (12) has shown that in tetanus and strychnine poisoning the coördinating mechanisms of the central nervous system are affected in such a manner "that in certain great groups of musculature the reciprocal inhibitions normally assured by the central nervous mechanism are changed into excitations." In regard to the similar action of these agents on the *cerebral hemispheres* this investigator says: "Little has met me in the course of observations on the reactions of the cortex under strychnine or tetanus toxin to indicate that the transformation of the motor effects of the reactions is due to the action of these agents on the cortex itself. The change of result seems quite explicable by alterations produced in lower centers, *e.g.*, spinal and bulbar, on which the cortex acts."

The opinion of Sherrington that strychnine converts inhibition into excitation has not remained unchallenged. Verworn and his pupils, notably F. W. Fröhlich, have instituted many experiments in opposition to this view. Verworn finds that strychnine actually facilitates (13) the appearance of certain inhibitions and Fröhlich (14) states that the conversion of inhibition into excitation observed by Sherrington follows as a result of the action of strychnine in increasing reflex excitability and that in Verworn's experiments also the favoring action of strychnine upon inhibition is similarly explainable. Bethe (15) has attempted a critical analysis of the complex questions here involved and presents certain criticisms which it would seem difficult for the Verworn school to meet satisfactorily. However these questions may be solved

ultimately, we think it is best for the present to state our findings in terms which do not commit us to an ultimate explanation of why a small dose of acid fuchsin which has no effect on an intact frog will at once throw a partially decerebrate frog into general convulsions.

The rapidity with which the convulsions follow upon the injury to the cerebral lobes under all circumstances makes it appear permissible to assume that the convulsions are held in check in the intact acid fuchsin frog as a result of inhibitory influences that proceed from the cerebral lobes to subcortical centers. We need not necessarily conclude that these inhibitory influences are of a tonic character, or in other words, that such impulses continually pass from the cortex of the frog to lower centers, though we believe in the general statement that inhibition in the central nervous system is an integral part of the activity of its irritable constituents. We are quite content for the present to confine the occurrence of such inhibitory impulses to the case of frogs poisoned with acid fuchsin and we are also prepared to believe that such influences may be ultimately derived from sensory paths. It has been repeatedly shown that "inhibition is elicitable" from the cortex, though, as Sherrington remarks, the real seat of the inhibition may lie in subcortical coördinating centers.

It has been shown that in frogs whose brains are intact larger doses of acid fuchsin and a longer time are required for the appearance of the convulsions. Under these conditions the drug tends sooner or later to "break through the barriers of inhibition" (Sherrington) on its own account, though here the heightened reflex excitability of the spinal cord may play a large rôle. The loss of inhibitory control may be traced in our "premonitory symptoms" especially when flexor spasms and flexor convulsions have made their appearance. At this time flexors respond to reflex stimulation while the extensors are still held in check by inhibitory influences. The flexors have normally a stronger tonus and normally respond more easily on direct as well as on reflex irritation to weak stimuli than the corresponding extensors, (16), and it is therefore not singular that they as well as their immediately controlling centers should more easily free themselves from inhibitory control.

It is also possible that the reflex centers here concerned are more quickly affected by the drug than those that are more concerned with excitation of extensor groups. At a later period when the reflex excitability has attained a higher level and the extensors also are freed from the inhibitory control of higher centers, general convulsions appear in which the stronger extensors overpower the flexors.

When, however, the anterior third of the cerebral lobes has been removed, excitation at once gets the upper hand as controlling or inhibiting influences are hereby removed. It now requires but little of the drug to break down the remaining barriers to the spread of reflex reactions to all parts of the skeletal musculature. *The slight operation on the brain so disturbs the normal ratio of excitation to inhibition that the former predominates. This unsuspected change in the state of the central nervous system is made evident only by the administration of a drug of the acid fuchsin type.*

CONCLUSIONS

1. Acid fuchsin produces in normal frogs (*R. pipiens* and *R. clamata*) general convulsions similar to those produced by strychnine. The dose required is 1.0 mg. per gram of body weight and upwards. Premonitory symptoms usually occur, namely, depressed irritability followed by heightened excitability and restless movements. These symptoms are succeeded by flexor spasms and flexor convulsions which in their turn give way to an extensor tetanus.

2. Fairly large doses (1 to 4 mgs. per gram of body weight) of the drug are required to produce convulsions. As a rule the tetanus will not appear until from one to twenty hours have elapsed, though in three instances out of forty-four the convulsions appeared in a half hour or less.

3. A state of fatigue induced by muscular exertion greatly hastens the appearance of the convulsions after acid fuchsin.

4. If the anterior third of the cerebral lobes be removed from frogs that have received the drug the convulsions will appear at once, the interval varying from one-half to thirteen minutes. The dose of the drug that is here required is small, 0.35 mg. per

gm. of body weight being sufficient to produce tetanus. Premonitory symptoms may occur as before and the flexor convulsions are often well developed, but as a rule these events are now crowded into a short interval of time preceding the extensor tetanus. The results are the same if the partial ablation of the brain is made seven days prior to the injection of the drug. This operation on the brain may also bring on a brief period of convulsions in frogs that have been suspended for some hours and that have received Ringer's solution in place of acid fuchsin.

5. It is assumed that inhibitory influences pass from the cerebral lobes to subcortical coördinating centers, in explanation of the fact that small doses of acid fuchsin which have no noticeable effect on intact frogs, will at once produce convulsions in those that have been deprived of the anterior third of their cerebral lobes.

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THE TOXICITY OF MARTIUS YELLOW AND SOME OTHER ANILINE DYES AND THE ENTRANCE OF DYES INTO CELLS

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PART I. THE TOXICITY OF MARTIUS YELLOW

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The toxicity of aniline colors is of importance from a hygienic standpoint and of much interest from the point of view of theoretical pharmacology. The use of these dyes as colors for candy, ice creams, butter, maccaroni, wines and other articles of food, and in articles of clothing coming in contact with the skin from which they may be absorbed or upon which they may act, makes it important that their toxicity should be determined.¹ The aniline dyes may also be used to study the manner in which substances get into cells. Fischel and Overton have used them for this purpose and the latter author has arrived at the conclusion that the solubility of the dye in lipoid determines whether it can or cannot penetrate cells.

Martius yellow, or dinitro-alpha-naphthol, belongs to the nitro colors and is called also naphthol yellow, naphthylene yellow, Manchester yellow, golden yellow and saffron yellow. It is also sold under the name of aniline yellow, the name of a totally different stain. My attention was called to it by Dr. Wiley, since it had been detected as a coloring matter in maccaroni shipped from St. Louis to Chicago which had been seized by the food

¹ For a review of this subject see Lewin: *Toxikologie*, 2d ed., 1897, p. 233.

inspectors for false labeling. At Dr. Wiley's request I tested the toxicity of the color.

This dye is generally regarded as poisonous. Its use is prohibited by the laws of Belgium, Italy, Switzerland and some other countries. Its toxic nature was first recognized by Cazeneuve and Lepine² in 1885. A dog of 7 kilos received each day by mouth 0.5 gram of powdered yellow. From the second day onward there was diarrhœa and the vomiting of brownish stuff; loss of appetite and thirst. From the fourth day the dog lay down and panted constantly. Temperature 41° C. The urine was colored and contained albumen. On the sixth day the temperature was 43° C and the animal died. "It is certain that the animal died of poison, and also that he died of much less than that given him, since from the second day onward he vomited each day." Intravenous injection of .03 to .06 gram per kilo into dogs of 10 to 25 kilos was invariably fatal; producing a marked rise of temperature and rapid and forced respiration. In one case the temperature rose to 44° C. The animals died in $\frac{3}{4}$ to 1 $\frac{1}{2}$ hours from the time of the injection. A dose of 0.01 gram per kilo produced the same symptoms, but the animals recovered. Their conclusions are: "We conclude that dinitro-naphthol (neutral) is endowed with a very great toxicity, since it produces, in relatively feeble doses, a panting respiration, a great elevation of temperature and death."

Essentially similar results were obtained by Weyl.³ He describes several experiments in which the stain was given both by hypodermic injection and by feeding to dogs. When given either way, the color caused vomiting, diarrhœa, fever, rapid respiration and albuminuria.

The injections were followed by the death of the animal if sufficient was given. The amounts actually ingested were not determined in the feeding experiments, since the vomit and feces removed much of the stain. Small doses of 0.1–0.2 gram were fatal if repeated on several successive days. Weyl concludes: "Martius

² Cazeneuve and Lepine: *Compt. Rend. de l'Acad. des Sci.*, 101, 1885, p. 1167.

³ Weyl: *Sanitary Relations of the Coal-Tar Colors*, p. 95.

yellow, therefore, belongs to the injurious colors. As a coloring matter for food or drink its use should be wholly prohibited."

Two cases of poisoning have been reported: Diedrich⁴ reports a case in which a family became ill after eating food in which no toxic substance could be found except Martius yellow. In another case⁵ a sailor, fifty years old, took 90 (?) grams of Martius yellow. One hour later he vomited after drinking some milk. At 10 o'clock his skin was yellow. At 11:45 he entered the clinic. He was fully conscious; the heart active and strong; he was very restless. A few minutes later he died suddenly. Shortly after death there was a universal muscle contraction and extreme rigor. The urine taken from the bladder had an alkaline reaction, contained albumin and was of a yellow color. There was no methemoglobin and no reaction for sugar. The muscle rigor persisted for many hours. There were hemorrhages in the pericardium. The stomach showed hemorrhagic gastritis.

As the only accurate observations on animals are those of Cazeneuve and Lepine and Weyl, it seems worth while to put on record the following observations:

Several different samples of stain were obtained. One of Grüber's stain called Martius yellow; two one-pound packages obtained from Sargent in this city and labeled naphthol yellow, one manufactured by the Badische Company, and the other by the Hudson River Aniline Works; one by the Berlin Aniline Works, labeled Martius yellow; one made by the Badische Company labeled Martius yellow; and one of the Badische Company, labeled aniline yellow which was not used in this work. The specimens used possessed the physical, chemical and physiological properties of Martius yellow, and in the following experiments no distinction will be made between them. In some experiments one sample, in other experiments other samples were used.

For a part of the experiments the stain was prepared by dissolving in hot water, adding a little hot sodium oxalate solution

⁴ Diedrich: *Zeitschrift für Untersuch, Nahrungs u. Genussmittel*, vol. v, p. 364, 1905.

⁵ Abstract in *Virchows Jahresbericht*, 1893, i, p. 445.

to precipitate the calcium and make the sodium salt, filtering off the calcium oxalate from the hot solution and purifying the stain by precipitating it two or three times with sodium chloride and then recrystallizing it from hot water twice or thrice. It crystallized out as very fine thin long needles. In part of the experiments the oxalate treatment was omitted and the recrystallized stain was fed by mouth or injected subcutaneously. Both samples showed the same toxicity to frogs and dogs.

Several frogs were injected under the skin of the back with a solution of the stain in water. The following results were obtained:

Frog 1. Weight 33 grams; .002 gram in 20 minims of solution in the dorsal lymph sac at 2.50 p.m. The frog gave evidences of pain at the point of injection. At first he jumped about violently, then tried to wipe off the stain with his foot and then sat with his head down and his eyes shut. The flank respirations became very marked and dyspnoëic, increasing from 12 to 78 per minute. Reflexes diminished very soon and at 3.15 there was no response on pinching the toes. The frog lay prone and unable to move. Respiration stopped at 3.25, 35 minutes from the injection. The heart was still beating very slowly when the frog was cut open at this time.

Frog 2. Weight 35 grams; .0026 gram of stain. Same symptoms. Reflexes abolished in 15 minutes. Respirations stopped in 17 minutes; heart stopped shortly after in diastole.

Frog 3. Weight 30 grams; dose .0039 gram under abdominal skin. Same symptoms. Respiration stops in 32 minutes after injection. Reflexes gone in 30 minutes. Heart stops in diastole.

Frog 4. Weight 26 grams; dose .0035 gram. Same symptoms. Respiration stops in 25 minutes.

Frogs 5, 6, 7. Weight about 30 grams each; dose each .002 gram in dorsal lymph sac. Dead in 25 minutes with same symptoms.

Frogs 8 and 9. Weight 30 and 28 grams; received between .001 and .002 gram in dorsallymph sac. A part of the stain was not in solution. Both apparently dead in 45 minutes. They were kept moist two days, but did not recover.

From these experiments it may be concluded that 2 mgs. of the stain under the skin of the back will kill a 30 gram frog in 30

minutes under the symptoms of excitation, marked dyspnoea and loss of respiration and paralysis. Fatal dose 60 mgs. per kilo.

Guinea pig 1. Weight 242 grams; dose .0033 gram subcutaneous. No result except quickened respiration, which rose to 134 per minute.

Guinea 2. Weight 200 grams; injected subcutaneous .0038 gram. No result.

Guinea 3. Weight 233 grams; 24 mgs. injected under skin of abdomen. Not all of stain in solution. Injected at 8.55 a.m. Respiration before injection 80 per minute. On injection the guinea squealed and showed evidences of irritation at the point of injection. At 9.10 respirations 180 per minute, deep and occasionally gasping. At 9.15 falls on one side. At 9.19 dead and in rigor mortis, the muscles becoming stiff at once. The left heart was in systolic contraction. The right had some blood in it. A small trace of color in the intestine.

Guinea 4. Weight 228 grams; 24 mgs. of stain under the skin of the side at 8.57. Not all stain in solution. At 9.12 respirations 112 per minute; at 9.35, 117 per minute, gasping and labored. At 9.40 falls on side gasping and at 9.45 is dead and in rigor mortis. Heart in systolic contraction. No color in intestines.

Guinea 5. Weight 274 grams; 48 mgs. under skin at 8.59. At 9.15 respirations 170 per minute, labored. At 9.21 gasping, lying on one side. At 9.25 dead and in rigor at once. Left heart contracted. No methemaglobin in the blood. No color in intestines.

Guinea 6. Weight 212 grams; well fed; received .012 gram suspended in 3 cc. of water subcutaneous. Not all dissolved. No result except slight quickening of respiration.

Guinea 7. Weight 230 grams; dose .02 gram subcutaneous. A part only dissolved. Respiration rose from 80 to 130 per minute. Recovery.

Guinea 8. Weight 223 grams; received .008 gram subcutaneous. No result.

From these experiments it appears that 80 to 100 mgs. of stain per kilo are fatal for guinea pigs in 15 to 30 minutes when injected subcutaneously. Not all the stain was in solution in these cases. The symptoms are: great acceleration of respiration; systolic contraction of the heart; immediate rigor of the muscles on death.

Experiments on Dogs

Dog 1. A small, thin puppy. Weight 2400 grams. He received 10 cc. of an aqueous solution containing 0.024 gram of stain subcutaneously at 9.40, May 9th. At 9.50 he swallows repeatedly and drinks freely. He will not eat. Respiration becomes more rapid. Drinks. At 10 a.m. defecates. Gait uncertain. At 10.03 vomits violently and repeatedly. Eyes widely dilated. 2 p.m. lies curled up. Will neither eat nor get up. Lies this way two days refusing all food and dies on the third day. Dose 10 mgs. per kilo. This dog was evidently unusually sensitive to the poison. He was not a strong puppy at the start.

Dog 2. A strong, active fox terrier. Weight 8400 grams. Put in cage 2 p.m., May 9th. Urine collected and found to be normal acid in reaction and free from albumen. At 1.55 p.m., May 10th, fed 0.5 gram of stain in a small piece of meat. May 11th, defecated dark feces during the night, rather soft. Not ill. Not very hungry but he ate some meat with 1 gram of stain at 8 a.m. At 9 a.m. has had two yellow diarrhoeic movements. At 9.40 dog panting as if very hot. Tongue out. Respirations over 200 per minute. Room temperature 18° C. Nose hot. Drinks and urinates frequently. Urine orange colored. Anal temperature 40° C. 10.22 vomits freely a yellow vomit. Gait irregular. Eyes bright, widely dilated pupils. Very rapid panting continues until his death. At 1 p.m. too weak to stand. Hind legs paralyzed. At 1.25 found dead in the cage and in a strong rigor mortis. Autopsy at once. Intestines and pancreas congested and very hot to the touch. A thermometer slipped under the liver registered 43.5° C. Blood contained no methemoglobin. Heart in systolic contraction. The urine collected from this dog passed on May 10th–11th showed the presence of a small amount of coagulated precipitate not soluble in alcohol or ether, when heated with a drop of acetic acid. Nitric acid test positive. Albumin present in small amounts. The urine was strongly alkaline in reaction and on adding acid effervesced freely.

Dog 3. Male. Weight 6400 grams; subcutaneous injection 0.048 gram, not all dissolved (7 mgs. per kilo). No effect except

slight diarrhoea and loss of appetite. Next day did not eat. The second day after he eats .2 gram of the stain in a croquette. Eyes dilated somewhat and respirations a little quickened, but no other effect except a profuse and watery, yellow diarrhoea.

Dog 4. Female. Weight 7200 grams. Black and tan. Subcutaneous injection of .048 gram only in part dissolved. Next day, May 11th, very watery, yellow diarrhoea. On May 13th gave her about .75 gram of stain in her food. Within the hour vomits much of this. Diarrhoea. Eyes dilated. May 14th apparently normal. Gave .4 gram in food. Vomits and defecates freely, and at 2 p.m. nose hot, sits panting as if in fever. Final recovery.

The experiments on dogs confirms those on frogs and guineas in showing the toxicity of the dye. It produces a very great rise in temperature, which with the intense contraction of the heart may be the cause of death, rapid respiration, vomiting and defecation, dilation of the pupils and in fatal cases the immediate onset of rigor mortis. The fatal dose for dogs was 10 mgs. per kilo. in dog 1. This, however, was a weak dog and a puppy. For healthy adults the dose is larger. These experiments do not permit a statement since so much of the stain was vomited, when given by the mouth. Cazeneuve and Lepine, however, found the fatal dose producing death in half an hour, to be between 30 and 60 mgs. per kilo when given intravenously.

This stain is therefore very toxic. It is about one-fourth as toxic as dinitro-cresol or Victoria yellow. Weyl⁶ found for dogs a fatal dose of this poison was about 15 mgs. per kilo subcutaneously. Sollman⁷ states that the fatal dose of cocaine hydrochloride for frogs is about 60 mgs. per kilo, and for dogs about 70 mgs. per kilo hypodermically. These figures correspond closely to those of Martius yellow. We may therefore say that toward these animals the stain is as toxic as cocaine. If a man is as sensitive kilo for kilo as a frog, the fatal dose for a man would be about 4.2 grams, for a man of 70 kilos. If, however, man is as sensitive to the stain as he is to cocaine, the fatal dose should be somewhat more than .2 gram when taken hypodermically.

⁶ Weyl: *loc. cit.*

⁷ Sollman: *Text Book of Pharmacology*, 2d ed., 1906, p. 950.

In its toxicology this stain resembles cocaine in many particulars. Both drugs dilate the pupil; both cause a great rise of temperature and marked acceleration of respiration; both are general protoplasmic poisons. Both annihilate the irritability of muscles and nerves. Both act strongly on the heart. In some particulars they differ, however. The stain probably possesses an irritant, instead of an anesthetic, local action. Cocaine produces convulsions which are absent here; and cocaine does not produce so marked an effect in bringing on muscle rigor and causing systolic standstill of the heart. In its action on temperature Martius yellow strongly resembles its near relative, tetra-hydro-naphthylamine,⁸ which also causes a marked rise in temperature and rapid respiration and dilation of the pupil. It appears to differ from such nitro compounds as nitro-cresol or nitro-benzol in its smaller liability to produce methemaglobin and also in the absence of convulsions. This may be due to the toxic action of the Martius yellow on the heart causing systolic standstill before a convulsant dose is reached. The fact that the reduced body, tetra-hydro-naphthylamine, produces the same symptoms as Martius yellow, suggests that they both get turned into the same substance in the organism. Mr. Tashiro has suggested that the active principle may be the intermediate substance, the naphthyl-oxime.

PART II. THE TOXICITY OF VARIOUS ANILINE DYES TOWARD THE COLONIAL INFUSORIAN, VOLVOX GLOBATOR

ELIZABETH LONGFELLOW

The studies were carried out at Wood's Hole in the summer of 1906.

The object of this investigation was to get some information of the relative molecular toxicity of various dyes, for the sake of obtaining additional data on the determining causes of toxicity. The animal chosen, a colonial infusorian, is large enough to be conveniently handled and it could be obtained easily in large

⁸ Stern: Virchows Archiv, cxv, 1889, p. 14.

numbers from a pond near the laboratory. It swims slowly but persistently by means of cilia or flagella and the action of the stain may be watched under the microscope. The action on the cilia was chosen as the point to be studied, and the concentration of each stain was determined which would stop movement in a certain time. By using this animal errors in toxicity due to lack of absorption are avoided or reduced, since the cilia project freely into the water.

All the cultures of the *Volvox* were brought from the same pond but for unknown reasons the organisms at different times varied somewhat in their power of resistance. The stains were Grüber's of Leipzig, and were employed without further purification.

In each experiment five cc. of water containing a considerable number of *Volvox* were taken and to them were added by means of a pipette graduated to hundredths of a cc. .1 to 1 cc. of the stain solution made to a known strength. The stains were generally made up in m./125, or m./250 solutions, and diluted from this. There was a good deal of variation in different colonies in their power of resistance. In all cases, however, the colonies were observed until motion of all had ceased and the figures in the last column of the tables indicate the time elapsed after the stain was mixed with the *Volvox* culture until all motion had ceased. In some cases motion ceased in nearly all the colonies long before it entirely ceased in one or two of them, but in all cases the time was taken for the most resistant. Thus in some cases nearly all the colonies might be motionless in 15 minutes, but a few continued for 30 or even 40 minutes. Some irregularity in the tables here and there is to be ascribed to the animals being more resistant in one case than another. Many more experiments than those given in the tables were tried, but for the sake of brevity have been omitted.

The toxicity of some of the basic dyes is remarkable. Thus toluidin blue and crystal violet are only a little less toxic than silver nitrate, the most toxic of all salts. It has been shown⁹ that

⁹ Mathews: Biological Studies of Pupils of Wm. T. Sedgwick, 1906, Boston, p. 108.

TABLE 1
Toxicity of basic dyes

DYES.	CONCENTRATION.	LENGTH OF TIME MOVEMENT CONTINUES IN THE DYE.
Crystal violet.....	$\left\{ \begin{array}{l} \text{M/ 50,000} \\ \text{M/100,000} \\ \text{M/400,000} \\ \text{M/800,000} \end{array} \right.$	Instant cessation 15' 60' 21 hours
Toluidin blue.....	$\left\{ \begin{array}{l} \text{M/ 50,000} \\ \text{M/200,000} \\ \text{M/400,000} \\ \text{M/800,000} \end{array} \right.$	Instant cessation 4' 8' 65' a little movement
Victoria blue.....	$\left\{ \begin{array}{l} \text{M/ 50,000} \\ \text{M/100,000} \\ \text{M/200,000} \\ \text{M/400,000} \end{array} \right.$	1' 16 $\frac{3}{4}$ hours 16 $\frac{3}{4}$ hours Survive
Methylene blue.....	$\left\{ \begin{array}{l} \text{M/ 25,000} \\ \text{M/ 50,000} \\ \text{M/100,000} \\ \text{M/200,000} \\ \text{M/400,000} \\ \text{M/800,000} \end{array} \right.$	2' 4' 20' 20' 20' 75'
Dahlia.....	$\left\{ \begin{array}{l} \text{M/ 6,250} \\ \text{M/ 12,500} \\ \text{M/ 25,000} \\ \text{M/ 50,000} \\ \text{M/100,000} \\ \text{M/400,000} \end{array} \right.$	2' 12' 40' 90' 120' 43 hours still living
Magdala red.....	$\left\{ \begin{array}{l} \text{M/ 60,000} \\ \text{M/ 12,000} \end{array} \right.$	Almost instant cessation Almost instant cessation
Neutral red.....	$\left\{ \begin{array}{l} \text{M/ 2,100} \\ \text{M/ 3,125} \end{array} \right.$	10' 18'
Thionin.....	$\left\{ \begin{array}{l} \text{M/ 1,500} \\ \text{M/ 2,100} \\ \text{M/ 3,000} \\ \text{M/ 6,500} \\ \text{M/ 12,500} \\ \text{M/ 25,000} \\ \text{M/ 50,000} \end{array} \right.$	3' 5' 25' 2 hours 2 hours 3 hours Live many hours

Toxicity of acid dyes

DYES.	CONCENTRATION.	LENGTH OF TIME MOVEMENT PERSISTED IN DYE.
Erythrosin.....	M/ 2,150	40'
	M/ 3,250	40'
	M/ 6,500	40'
	M/ 186	25' (A different lot of Voif- vox from preceding)
	M/ 500	43'
	M/ 812	80'
	M/ 1,625	4 hours
Eosin.....	M/ 125	21'
	M/ 520	3 $\frac{3}{4}$ hours
	M/ 1,050	3 $\frac{3}{4}$ hours
Congo red.....	M/ 343	16 hours
	M/ 800	35 hours
	M/ 3,000	71 hours alive
Methyl blue	M/ 750	29 hours
	M/ 881	65 hours
	M/ 2,083	89 hours
	M/ 3,125	89 hours
	M/ 6,250	89 hours still living
Bordeaux red.....	M/ 438	90'
	M/ 750	20 hours
	M/ 1,250	68 hours
Indigo carmine.....	M/ 1,562	96 hours still alive
Acid fuchsin.....	M/ 70	29 hours dead
	M/ 125	18 hours dead
	M/ 350	74 hours still vigorous
Carminic acid.....	M/ 875	114 hours, some still alive
Orange G.....	M/ 84	29 hours dead
	M/ 312	69 hours dead
	M/ 500	Live many days

an m./300,000 solution of this salt will stop the swimming of Volvox in 2 minutes. For cadmium chloride to stop in the same length of time takes an m./500 solution. In fact the basic dyes possess for these organisms a toxicity of the same order as silver nitrate, mercuric chloride and copper sulphate. The basic dyes vary a good deal in toxicity although they seem to penetrate cells with about the same ease. Thus thionin is roughly about 1/200 as toxic, molecule for molecule, as toluidin blue. Neutral red, the least toxic of the basic dyes examined, is about half as toxic as thionin, or at least in the same strength of solution, the organisms live about twice as long in the red dye as in the thionin. These results correspond fairly well with the result of Fischel¹⁰ who worked on the *intra vitam* staining with tadpoles. He found, also, that crystal violet and others of the basic stains were very toxic and that neutral red was the least toxic of all.

The acid stains are throughout far less toxic than the basic, as Fischel and others also found. However, eosin and erythrosin do not lack very much of the toxicity of the less toxic dyes of the basic class, such as neutral red. The sulfonated dyes are all less toxic than the non-sulfonated. Thus indigo carmine, acid fuchsin and orange G can hardly be called toxic substances toward these organisms, since they live in strongly colored solutions almost as well as in water. It is significant that eosin and erythrosin, the most toxic of the acid dyes tried, are the non-sulfonated dyes, eosin being the potassium salt of the tetra-brom derivative of fluoresceine and erythrosin, the tetra-iodine derivative.

The greater toxicity of the basic dyes has been ascribed to the greater ease of penetration into cells, but while this may be a factor, we agree with Fischel, that it is not the main factor, since the acid dyes will also penetrate. Moreover there is a great difference in toxicity between different basic dyes although, as has been said, they enter the cell with what seems to be the same speed. The toxicity of the dyes, as of all other substances, we believe to be correlated in most cases with two factors, *i.e.*, instability of the substance in the protoplasm, and its content of energy. At-

¹⁰ Fischel : Anatomische Hefte, vol. xvi, 1901, p. 415.

tention has already been drawn¹¹ to this factor as the determining factor of toxicity in the metals which are also basic substances, and we have no doubt that it determines the relative toxicity of the basic dyes also. Sulfonation renders the stains less toxic, in part perhaps because it makes them electro-negative and thus may affect their penetrating power, but mainly because of the greater stability of the sulfonated salt. Thus naphthol yellow, a non-sulfonated acid dye, is very unstable and very toxic; by sulfonation, although it remains an acid dye, its stability is greatly increased and its toxicity reduced. Evidently stable sulfonated colors should be preferred for addition to food stuffs, if any colors are to be added.

The greater activity of methylene blue, which is the tetramethyl-thionin, than of thionin itself is noticeable and again is correlated with a greater molecular instability, since methylene blue decomposes spontaneously in aqueous solution and more rapidly in the presence of the alkali. Evidently the free base is more toxic than the salt.

As some of these dyes have toward this organism a toxicity closely approaching that of corrosive sublimate, their addition to food stuffs should be totally prohibited.

PART III. THE PENETRATION OF ANILINE DYES INTO CELLS

A. P. MATHEWS

The method in which dyes and other substances penetrate cells has attracted of recent years a good deal of attention and is a matter of much interest. Overton¹² has particularly studied this matter of the penetration of dyes. He was at first of the opinion that only the free base, which has some solubility in lipoids, was able to penetrate the cell membrane which he, in common with others, regarded as of a lipid nature. The free

¹¹ Mathews: *loc. cit.*

¹² Overton: *Vierteljahresschrift der Natur-forsch. Gesellsch. in Zürich*, xlv, 1899, p. 88.

base was supposed to be formed from the salt of the dye by hydrolytic dissociation. Observations made more recently forced him to abandon this assumption. He made an extended study of the solubility of stains in benzol solutions of lecithin and cholesterolin and of the power of emulsions of lecithin to take up dyes from aqueous solutions. He found that a parallelism existed between the solubility of the stain in these solutions in lecithin and their power of entering cells. Thus all the basic stains were rapidly taken up by a benzene solution of lecithin, and to a less extent by such a solution of cholesterolin, whereas the acid dyes were taken up almost not at all. He concluded that these facts strongly confirmed his earlier opinion that the membrane of cells was composed of a lecithin-cholesterin layer, and that the solubility of substances in this layer determined whether the substance could enter the cell. Quite a different view was taken by Fischel,¹³ who investigated the power of dyes to stain *intra vitam*. He was of the opinion that the stain probably entered by combining with some of the elements of the cell. In other words Fischel, finding that all the basic dyes stained, although with varying power, while acid stains did not, laid stress on chemical combination rather than on solubility in a membrane as the determining factor in their penetration. Ehrlich's view of the power of toxins to act on cells is that the toxin or stain enters into a chemical combination with the membrane rather than goes into solution in it. R. S. Lillie¹⁴ in his extensive and thorough studies on the nature of contraction and the action of salts and other substances on contractile tissues and the formation of the fertilization membrane of eggs appears to adopt Overton's hypothesis, since he constantly speaks of the change in permeability of the membrane, as if substances passed through the membrane without combining with it, and elsewhere he mentions the fat or lipid solubility as determining the action of substances. Recently Robertson¹⁵ has criticised Overton's views of lipid solubility, and pointed out that stains

¹³ Fischel: *loc. cit*

¹⁴ Lillie: *American Journal of Physiology*, xxvi, 1910, p. 113.

¹⁵ Robertson: *Journal of Biological Chemistry*, iv, 1908, p. 1.

will unite with protein, thus confirming the work done by Heidenhain and the author.¹⁶ He throws doubt, also, on Overton's results with lecithin by stating as a result of some experiments of his own that the pure basic dyes such as methyl green will not stain lecithin dissolved in benzol. The staining Overton obtained, he suggests, was due to methyl violet.

It seemed worth while, in view of this difference of opinion, to go over the matter again. I have accordingly repeated some of Overton's experiments and added a few of my own.

In the first place, Overton is in error in my opinion in supposing that only lipoids will dissolve or combine with the basic dyes. I showed many years ago, and Heidenhain and Robertson and others have confirmed the observation, that the basic dyes will unite with albumin provided the reaction is alkaline. With modern knowledge of the chemistry of the albumins and colloids this amounts to saying that the basic dyes form salts with electro-negative albumin. Electro-negative albumin is nothing else than a salt of the albumin in which the albumin is functioning as an acid. In other words, since the proteins in the cell are certainly to some extent present as salts, when the stain enters we have a reaction of this type: $\text{Na Proteinate} + \text{Methylene blue chloride} = \text{Methylene blue proteinate} + \text{sodium chloride}$. It is, therefore, clear that the stain could as easily enter the cell in this way as by union with lecithin. Hence the fact that stains enter cells and happen also to be soluble in lecithin is no evidence that the cell membrane is lipid in nature. I agree with Robertson on this point. The basic stains would enter whereas acids do not, because they would form such a union with the protein and the acid stains do not. On the other hand, this does not permit us to affirm that the limiting membrane is of a protein rather than a lipid nature, if indeed there is such a membrane different from the protoplasm behind it. The question is left undetermined so far as this point is concerned.

While the stains thus unite with protein the question arises whether Overton's observations are correct, as to the solubility

¹⁶ Mathews: American Journal of Physiology, i, 1898, p. 445.

of the dyes in lecithin. This point was doubted by Robertson in a brief note at the end of his paper. It seemed to me that Overton's observations that the dyes colored the lecithin were convincing, but I repeated some of them and, as far as they go, my results confirmed him. Some lecithin was prepared from the star-fish eggs by alcohol-ether extraction and precipitation with acetone. The precipitation was repeated several times, allowing the ether solution to stand in the ice box each time for several days to settle out a white substance which was filtered off. By this means a light yellow, waxy lecithin was prepared which was probably still impure. It took up water more readily than egg lecithin, and emulsified easily. An emulsion was prepared by shaking and to this emulsion was added a little of the solution of the stain. It was observed, by examining under the microscope, that the drops of lecithin had colored themselves always by taking up the stain from the water. They always colored in the basic dyes, but never in acid dyes. Furthermore, an emulsion was prepared by rubbing up together lecithin, a little linseed oil and egg-white. This emulsion looks very much like protoplasm. Particularly the little granules of lecithin look exactly like the small granules in the star-fish egg. They stain like them, also, taking up the basic stains with avidity. In methylene blue, the granules take first a more violet tinge, due probably to methylene azure, for which they seem to have a greater affinity, but they afterwards become more blue. The fat drops would not stain. The protein, however, did stain to some extent, but not so deep as the lecithin. There is no doubt that Overton was right in his observations.

But when we come to consider the explanation of these facts of the absorption of stain by lecithin, I agree with Robertson, that it is far better to consider them as combinations rather than solid solutions, as Overton does. Lecithin is a salt. It exists in the cell, at least in part, as a salt.

It reacts with the stains, just as it reacts with metals, forming salts. This is the cause of its staining. The reaction is similar to that of protein. Methylene blue chloride + sodium lecithinate gives methylene blue lecithinate and sodium chloride. While the author believes that it is probably true that all true solutions

are in the nature of chemical combinations between solvent and solute and so there is in the last analysis no discrepancy between the two statements, yet as long as it is not generally admitted that solution is a chemical combination and the two words convey a different impression, it is better, in my opinion, to consider the stains as entering into a chemical combination with the membrane, rather than to state that they are in solution in it. Certainly, whatever the nature of solutions, there is no doubt that the stains combine with protein, lecithin, cholesterin and fatty acids as well as other constituents of the cell. There is in fact no more reason to speak of the stain as dissolving in the lecithin, than there is to speak of the sodium or potassium, which is in salt combination with the lecithin, as being in solution in the latter.

That the stains really combine with the lipoids is shown, also by the nature of the solvents in which they are supposed to dissolve. These solvents are one and all of them weak acids. Thus Overton found that the phenols, thymol, carvacrol, other aromatic alcohols and such alcohols as amyl, cholesterin, etc., dissolve the basic dyes. It is noteworthy that as soon as the acid nature is neutralized by tying up the free hydroxyl group in an ester combination, the stain no longer dissolves, or dissolves less easily. It is surprising that this fact did not suggest to Overton the chemical union rather than the solubility theory. Some of the aldehydes also dissolve the basic dyes. But in this case we are dealing again with weak acids, for the aldehyde group adds water and goes over, when in solution, in part into two alcohol groups. Overton's facts, therefore, show to my mind that weak acids (and alcohols) will dissolve basic dyes and since they do not dissolve acid dyes it seems clear to me that the dye forms a salt or ester with the alcohol or the acid.

This may be shown also, by shaking the dyes with oleic acid and water, instead of tri-olein and water. Oleic acid is stained at once and deeply by all the ordinary basic dyes, whereas the olive oil is not stained at all or but very faintly. However, if oleic acid is added to the olive oil then it becomes capable of taking up some of the dye. If the aqueous solution is dilute, the oleic acid may extract most of the color from the water. Here

again it is seen that weak acids dissolve the basic dyes by combining with them. The solubility of the dyes in ethyl acetate may be due to a slight hydrolysis of the ester and the combination of the dye with both the alcohol and acid.

We conclude then that the basic stains enter the cell by combining with the substances of the protoplasm, at first with the substances in the peripheral layer such as lecithin and the electro-negative-proteins, soaps, and possibly other substances. They are able to accumulate in the cell because they form insoluble compounds with the granules or other constituents of the protoplasm. Fat, or lipid solubility, is not a proper way to designate this union, unless it is understood that solution is in reality chemical combination. There is no evidence that solubility in lipid has anything to do with the penetration of substances into cells, but on the contrary a great mass of evidence against this view. In my opinion power of combining with the substances in the protoplasm generally determines in large measure the penetration of substances into the cell.¹⁷

CONCLUSIONS

1. Martius yellow, or dinitro-naphthol, a stain sometimes used in food products, is a very toxic substance. The fatal dose for frogs is 60 mgs. pro kilo; for guinea pigs 60–100 mgs. pro kilo; for dogs, intravenously, 30–60 mgs. pro kilo.

2. It causes vomiting, diarrhoea, albuminuria, a great rise of temperature, great increase in respiration, dilation of the pupil and sudden death, presumably by systolic standstill or spasm of the heart. The muscles of mammals killed by the drug go immediately on death into a strong rigor, which they maintain long after death. The stain is as toxic for frogs and dogs as cocaine and resembles this drug and tetra-hydro-naphthylamine in many of its actions.

¹⁷ Since sending this paper to the printer I have become acquainted with the fine paper of W. Ruhland (*Beiträge zur Kenntniss der Permeabilität der Plasmahaut. Jahrbücher für wissenschaft. Botanik, vol. 46, 1908-9, p. 1*). My work, as far as it goes, is simply a confirmation of his.

3. The molecular toxicity of many acid and basic stains toward the infusorian, *Volvox globator*, was studied. All basic stains are more toxic than the acid stains studied. Toluidin blue and crystal violet were most toxic, being for these organisms as toxic as mercuric chloride and silver nitrate, the most toxic of all salts. *Dahlia*, thionin and neutral red were less toxic. The last is little more toxic than eosin and erythrosin.

4. The sulfonated acid stains are less toxic than the non-sulfonated. Orange G, carminic acid and acid fuchsin were almost entirely harmless.

5. The greater toxicity of the basic as compared with the acid stains and of the non-sulfonated as compared with the sulfonated acid dyes is probably due to the greater stability of the sulfonated and acid stains. Like all other substances, the more reactive they are, the more toxic they are. This result confirms an earlier paper on the cause of the toxicity of salts and other substances.

6. The basic stains combine readily with lecithin. They do not, properly speaking, dissolve in it, as Overton thought, but form a salt combination with it. All basic stains tried stained oleic acid and lecithin. The penetration of the stain is in our opinion, as Fischel and Robertson maintained, by chemical combination rather than by solution, unless it be granted that solution is also chemical union.

PHYSIOLOGICAL STUDIES IN ANAPHYLAXIS. II.¹ REACTION OF SMOOTH MUSCLE FROM GUINEA- PIGS RENDERED TOLERANT TO LARGE DOSES OF SERUM

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Recently² it has been shown that smooth muscle contracts quite readily when exposed to small quantities of serum. Furthermore, this normal irritability may be greatly augmented by first sensitizing the animal as is done in studies of anaphylaxis. Smooth muscle from sensitized guinea-pigs, excised and treated with serum, records a contraction curve much greater in extent than does an unsensitized muscle preparation similar in every other respect. Since there is present this peculiar reaction of smooth muscle in sensitized animals it seemed probable that this supranormal irritability might be reduced to normal or subnormal if the animals were first rendered immune to relatively large doses of serum. Three methods of rendering the animals immune to the pharmacologic action of serum are suggested: (1) The animal may be injected subcutaneously with gradually increasing doses of serum, the doses being given at relatively short intervals of time, having the number of injections sufficient to make the total interval of time between the initial or sensitizing dose and the final dose sufficiently long so that whatever sensitization may be set up is neutralized by the subsequent injection of serum.

¹ Schultz, W. H.: I. The reaction of smooth muscle of the guinea-pig sensitized with horse serum. *Journ. Pharmacol. and Exper. Therap.*, 1910, I, p. 549.

² Rosenau and Anderson seem to be the first to have demonstrated this point. Rosenau and Anderson: Studies on hypersusceptibility and immunity. *Bull. Hyg. Lab., U.S. P.H. and M.H.S.*, 1907, April.

In this way by the end of 20 days doses large enough to kill a very sensitive guinea pig ought to have but little effect, either because of an acquired immunity or an acquired tolerance towards a foreign serum; (2) The animal may be sensitized in the usual way and after 20 days sublethal doses of gradually increasing size but small enough not to produce grave symptoms are given, thus gradually neutralizing that which is necessary to high sensitization; or, (3) highly sensitive animals may be injected with sublethal doses large enough to produce grave symptoms, still leaving them in a condition not to respond to other large doses of serum within reasonable lengths of time. If smooth muscle from animals desensitized by any of these three methods be tested it ought not to react like that from a highly sensitized guinea-pig provided the desensitization is complete and permanent. In other words, if there is a condition of absolute immunity set up when large doses of serum no longer kill, then muscle preparations from such animals ought to respond in a manner similar to that from normal ones, or possibly show even a subnormal irritability. It will be seen, however, that neither of these conditions obtains when the first method mentioned of rendering guinea-pigs tolerant to large doses of serum is employed.

In this series of experiments guinea-pigs of known descent, raised in our own pens, were used. A number of young pigs weighing from 180 to 250 g. were divided into three groups, *a*, *b*, and *c*, and kept in the same pen, bedded with hay, and fed on oats, cabbage, and water. Group (*a*) were reserved for normal controls, group (*b*) were sensitized by subcutaneous injections of 0.1 cc. of sterile horse serum, and group (*c*) were injected with gradually increasing doses of sterile serum, the doses for the first 21 injections being at two-day intervals. The injections were started with 0.1 cc. on July 1 and the dose doubled (0.1, 0.2, 0.4, 0.8, 1.6, 3.2) until 3.2 cc. was reached on July 14, after which 4 cc. was injected peritoneally until July 24 when the irritability of the various organs was tested. At the present time the results obtained with smooth muscle from the intestinal tract will be reported. As a rule each experiment consisted of from three to six tests with tissue from two different animals, one animal, the control, being

either a normal animal or a sensitized one, the other being a desensitized (tolerant) guinea-pig. The desensitized pig had been rendered so tolerant to serum as to react no more than does a nonsensitized animal injected intravenously with $\frac{1}{2}$ cc., or peritoneally with 4 or 5 cc. of serum. The following protocol represents more in detail one type of experiment:

Experiment 115, a and b, August 1, 1910

a = normal non-sensitized guinea-pig.

August 1. Wt. 321 gms., 10:7 a.m. 4 cc. intraperitoneal injection of horse serum. 12:51 p.m. decapitated. Small intestine tied off, excised, and placed in Howell's solution, through which washed oxygen was slowly bubbling.

Segments 35 mm. long suspended in saline bath and treated with serum. (See fig. 1.)

Experiment 115 b.

Guinea-pig injected with gradually increasing doses of serum.

July 1, Wt. 199 gms. Subcutaneous injections of sterile horse serum. July 1, 0.1 cc., July 3, 0.2 cc., and intraperitoneal injections July 5, 0.4 cc., 7th 0.8 cc., 9th, 1.6 cc., 11th, 2.0 cc., 13th, 2.5 cc., 15th, 3 cc., 17th, 3.5 cc., 19th, 4 cc., 21st, 23d, 25th, 27th, and August 1, 4 cc. respectively.

August 1, Wt. 315 gms. 115a and 115b, when injected intraperitoneally with 4 cc. of serum reacted about alike; without the cards it would have been impossible to distinguish the control from the desensitized animal, since both animals occasionally rubbed their noses, nawned their flanks, and yawned. Perhaps 115b was on the whole more drowsy, but 115a examined its abdomen more frequently. There were, however, no bucking movements, coughing or sneezing, no abnormal excitement followed by subsequent paralysis and respiratory symptoms known to be characteristic of anaphylactic shock. Only such symptoms were observed as are seen when normal animals are injected for the first time with large doses of serum.

August 1 at 12:49 115b was decapitated, the intestine tied off, excised, and placed in oxygenated saline and a 35 mm. segment suspended in saline and tested with serum (see fig. 1).

After this preliminary treatment segments of each of the excised intestines, as nearly alike as possible, were suspended in a saline bath kept at nearly a constant temperature. The light recording levers were also as nearly alike as possible and arranged to record the myogram of the control above or below that of the tested segment, a slowly revolving Harvard Kymograph being used in obtaining the records on smoked paper. In the course of the experiments it was soon learned that certain precautions, aside

from changes in temperature and rate of oxygenation had to be observed:

(1) The animals used for a given experiment had to be about the same size, for as is well known the size of the alimentary tract increases in proportion to the size of guinea pig, making the small intestine of a 500 gm. pig much heavier and stronger than that from a 300 gm. pig.

(2) Intestinal segments though taken from animals of approximately the same size must be in similar states of physiological activity. That is, both animals must be well and healthy, the stomach and intestine should contain sufficient food to insure the viscera of each being in about the same condition of digestion and absorption, since there is considerable difference in the extent of a myogram recorded by a segment excised during the height of the digestive period and in one taken during the fasting stage or period before feeding.

(3) Segments may be compared after standing in oxygenated Ringer for a number of hours. The best results are, however, obtained soon after bleeding the animal. Hence all segments were treated as soon as possible.

(4) Segments, in order to be comparable, must be taken from approximately the same level of the alimentary canal.

1. EFFECT OF SINGLE INJECTIONS OF SERUM UPON THE NORMAL IRRITABILITY OF THE INTESTINAL MUSCLE

A single intravenous injection of $\frac{1}{2}$ cc. of horse serum seems to have no effect upon the normal irritability provided the muscle be tested within a period of one or two hours after the injection. And probably has no effect until the process of sensitization sets in.

If, however, a sufficient time has passed since the first injection of serum, the intestinal muscle shows a marked increase of irritability, even though the animal, judged by the usual method, shows no, or at best very mild, symptoms of anaphylaxis. Just how long a time must elapse before this increased irritability can be detected has not been worked out, but it is probably coincident with the initial stages of anaphylaxis.

EXPLANATION OF FIGURES

FIG. 1. Small intestine from a nonsensitized guinea-pig and from a guinea-pig rendered tolerant to horse serum.

Experiment 115a and 115b. August 1, 1910. Curve 115a from intestine of non-sensitized guinea-pig weighing 321 gms. Ether anaesthesia. Intestine removed and placed in oxygenated saline 1:1 p.m. Segment 35 mm. long, taken from duodenal end about 12-15 in. from stomach, suspended in 10 cc. of Howell's solution at 36.4°, 1:19 p.m.; 1:23 p.m., 1 cc. horse serum (36.4°) added to saline bath. Ratio of lever arms 150 mm. to 30 mm. weight of 0.5 gms., 30 mm. from fulcrum on writing arm.

Curve 115b from 35 mm. segment of small intestine from a guinea pig tolerant to horse serum. Guinea pig treated as follows. July 1, weight 199 gms.; subcutaneous injections of horse serum July 1, 0.1 cc., July 3, 0.2 cc., July 5, 7, 9, 11, 13, 15 and 17, inclusive, intraperitoneal injections of 0.4, 0.8, 1.6, 2.0, 2.5, 3.0 and 3.5 cc. of horse serum respectively. On each of the following days 4 cc. of the same serum was injected intraperitoneally. July 19, 21, 22, 27, 29 and August 1. August 1 Wt. 315 g. 10:9 a.m. received last injection; pig normal, no abscesses, eats heartily. The larger doses of serum seem to affect this pig less than similar doses affected normal controls. 12:56 p.m., ether anaesthesia. Intestine removed and placed in oxygenated saline solution; 1:19 p.m. 35 mm. segment suspended in 10 cc. of saline at 36.4° C. along side of 115a. 1:23, 1 cc. horse serum (36.4°) added to the saline bath in same manner as to control.

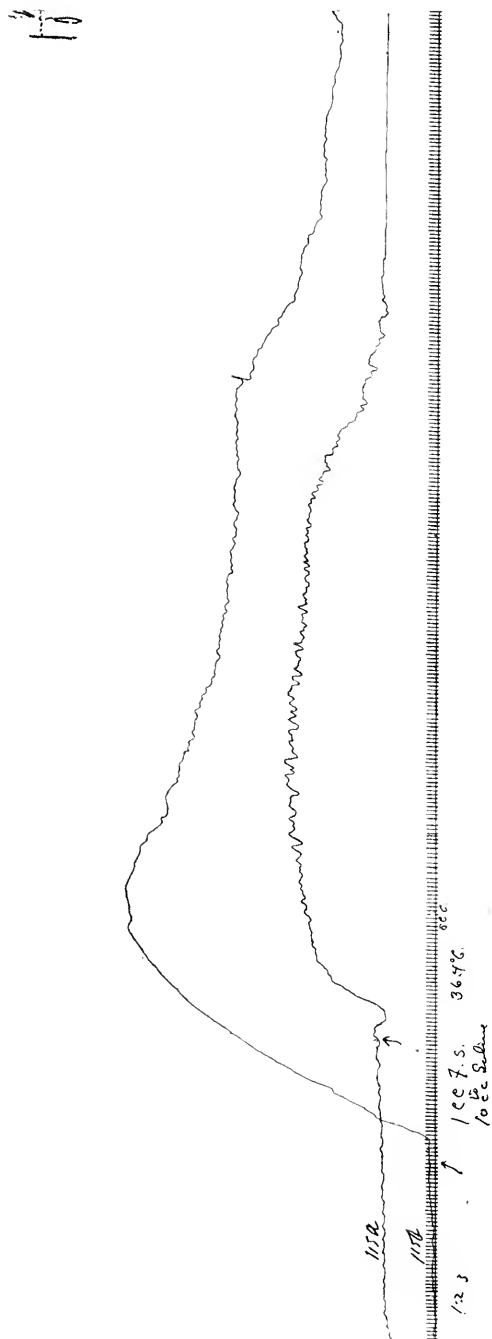


Fig. 1

2. THE EFFECT OF REPEATED INJECTIONS OF GRADUALLY INCREASING DOSES OF SERUM

It seems to be quite generally accepted that in order to produce in guinea-pigs a condition of anaphylaxis a certain interval of time must elapse after the sensitizing dose. So far as is known there are no abrupt changes resulting in complete anaphylaxis. The present data support the idea that the changes in irritability as tested by the second injection are gradual, finally reaching a maximum in from 10 to 20 days and then very slowly falling off. If the ordinates represent the degree of irritability and the abscissa the number of days since the sensitizing dose, a curve may be plotted representing the changes in irritability towards the second dose of serum. The ascending limb of such a curve will be gradual but much steeper than that representing the return to normal. Indeed, the descending limb remains, after the first preliminary drop, almost flat so slowly do the conditions responsible for anaphylaxis disappear. Now so far as the gross symptoms are concerned irregularities may be introduced into the irritability curve. For example, relatively large doses injected subcutaneously soon after the sensitizing dose greatly hinder the conditions necessary to a vigorous response of the organism to a subsequent injection of serum provided too long an interval has not elapsed between the last two doses. Whatever it is that is affected by these so-called immunizing² doses of serum one thing is certain, that there is a condition set up inhibiting either the extra or acquired irritability of the cells, or else neutralizing the medium through which they are stimulated. This is true of the intact animal, but is it true of tissues when removed from the body?

Figure 1 illustrates the results of a crucial test which ought to throw some light upon this vexing problem. In this series of experiments of which 115*a*, *b*, and *c* were members, *a* was a normal control, *b* injected with increasing doses of serum reacting less, when judged by the gross body symptoms, to the 4 cc. injections than to the 2 cc. ones; still never at the most did it show true anaphylactic symptoms, whereas *c*, the sensitized animal, died within 5 minutes from anaphylactic shock. Judging from the gross reac-

tion of 115*b* one would imagine the animal well protected against large doses of serum. The myogram, however, shows the smooth muscle to be still hyperirritable, in fact it reacts very much like that from a sensitized animal. It matters not whether the final dose of serum be injected into the abdominal cavity or into the circulation, for the muscle of this group of pigs reacted much as did that from sensitized animals. These results would seem to indicate that at least in animals tolerant to serum there is still present that hyperirritability of smooth muscle so characteristic in sensitized animals. Since the smooth muscle of the intestine is hyperirritable towards serum it would hardly be correct to say that there is not present at least a high degree of latent sensitivity. Yet by reason of the absence of the usual characteristic anaphylactic symptoms some chemical or physical process seems to be responsible for protecting the pig from the injection of what is ordinarily a lethal dose of serum.

CONCLUSIONS

1. Single intravenous or intraperitoneal injections of horse serum seem to have no effect upon the normal irritability of intestinal muscle towards the same serum provided the muscle be tested within a period of one or two hours after the injection.

2. If, after the first injection of foreign serum, a long enough interval elapse, intestinal muscle shows a supranormal degree of irritability towards the same serum. The time necessary to acquire this increased irritability is probably coincident with that required for the process known as sensitization in anaphylactic animals.

3. Guinea-pigs may be rendered tolerant to large doses of foreign serum by injecting increasingly large doses of it, at intervals of two days, for a period of 20 to 30 days. The gross body reflexes and the cardiac and respiratory reactions differ markedly from those of a sensitized animal, but intestinal smooth muscle continues to show a supranormal irritability towards a serum similar to that of smooth muscle from a sensitized animal.

4. The tolerance induced by repeated injections of foreign serum resembles tolerance acquired towards certain chemical substances familiar to Pharmacologists. As to immunity, it seems impossible by repeated injections to initiate a condition of absolute immunity, since certain tissues not only remain irritable to the serum but acquire a supranormal irritability to it.

THE ACTION OF ETHER ON AN ANAEROBIC ANIMAL TISSUE

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As the respiratory activity of nerve tissues is obviously of great importance to their proper functioning, it seems not unlikely that many of the substances which affect the nervous system may be acting on this fundamental process. As I knew of no observations upon the action of ether or other anesthetics on anaerobic animal tissues in the absence of atmospheric oxygen, I thought it worth while to see if ether would act in the same manner on such tissues as on aerobic. I accordingly tested the action of the drug on the nerve cord of the heart of the king-crab, *Limulus*. This is a ganglionated nerve-cord which furnishes the impulses causing the heart to beat. It is possible, as Carlson (1) has shown, to prepare the cord so that the drug comes in contact only with the ganglion and not with the muscle. Newman (2) found that the ganglion would continue to function with but little change in its activity for an hour or more in an atmosphere of hydrogen.

The experiments consisted in separating the nerve-cord from the posterior part of the heart, leaving it attached only to the front segment of the long, tubular heart. The cord was placed in a glass tube through which hydrogen, or hydrogen mixed with ether, or air could be passed at will. The anterior muscular segment continued to receive impulses from the nerve cord and continued to beat. It was suspended in sea-water and its contractions recorded on a drum. The method of preparation was essentially that employed by Newman (2). The hydrogen was generated by

pure zinc and sulfuric acid and the gas was well washed in alkaline and acid permanganate, sodium hydrate and water. A tracing was first taken while the ganglion was in air, then the air was replaced by hydrogen and after the ganglion had been in the hydrogen for from 10 to 20 minutes, the hydrogen was replaced by hydrogen mixed with ether. After the heart had been brought to rest by the ether, the hydrogen-ether mixture was washed out by hydrogen or air and the course of the recovery noted.

The results of the experiments may be seen in the tracings. They may be summarized as follows:

1. The replacement of air by hydrogen generally stimulated slightly both the rate and amplitude of the contractions. Newman (2). *Fig. 1, A.*

2. The contractions slowly diminished in the course of an hour in hydrogen from a rate of 17 to a rate of about 10 per minute. If air was then readmitted the rate usually fell still lower. *Fig. 1, B.*

3. Substitution of the ether-hydrogen mixture for hydrogen always caused a short preliminary increase in the rate and amplitude of the contractions and increase in the tone of the heart-segment, provided the ganglion was in good condition and had not previously been anesthetized. This preliminary stimulation did not occur, or lasted only for two or three beats if the ganglion had been anesthetized once or twice before. Further action of the ether in all cases caused a cessation of contractions. (*Fig. 1, C. and Fig. 2.*) This result is closely similar to the results obtained by Carlson (3) for the cyanide action on the ganglion.

4. When the ether was washed out with a stream of hydrogen, the ganglion was markedly stimulated on recovery, as shown by the increase of the rate and amplitude of the contractions above the rate and amplitude existing before the etherization. The ganglion generally recovered fully in the hydrogen. (*Fig. 1, D.*)

5. The recovery in the hydrogen was in most cases as prompt and complete as it apparently would have been in air; in a few cases, however, after being first for a considerable period in the hydrogen, the recovery from ether was very slow in the hydrogen, but rapid on changing to air. (*Fig. 3.*)

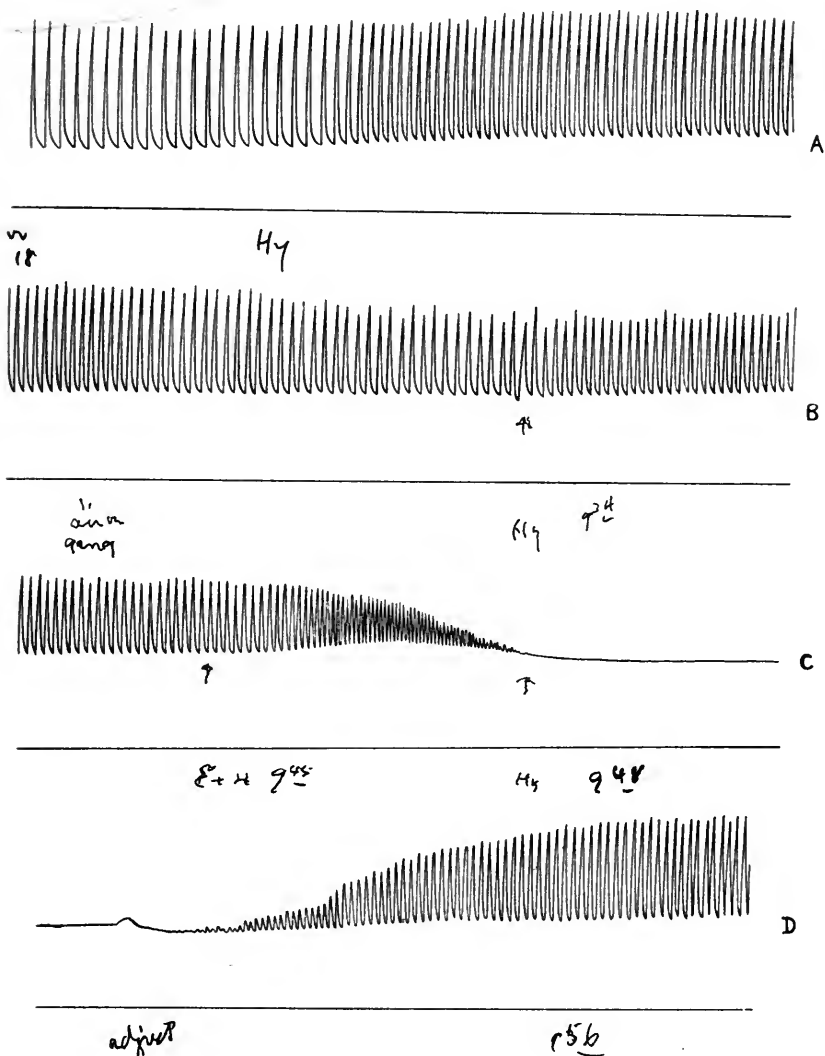


FIGURE I. Successive portions of a tracing from the fore segment of *Limulus* heart. *A*, Increase in rate, amplitude and tone of the heart when the ganglion is exposed to hydrogen. Hydrogen began to be admitted at *Hy*. *B*, The slowing and decrease in size of the contractions on readmitting air to the ganglion. At 9:34 hydrogen was again readmitted and the rate increased. *C*, The increase in rate and tone of heart when ether and hydrogen are supplied the ganglion at 9:45. At 9:48 ether was replaced by hydrogen. *D*, shows recovery and after-stimulation when the ether is removed by hydrogen. Time marker gives minute intervals. All tracings read from left to right. The above tracings and also those of the following figures were redrawn from the original tracings.

These results show, therefore, that the actions of ether on this facultative anaerobic tissue, while undergoing anaerobic respiration, is in all respects essentially the same as the action of ether on other aerobic nervous tissues, and as the action of ether on the same tissue in the presence of air, i.e., primary stimulation, followed by inhibition and after-stimulation on recovery.

These results considered by themselves might have at least two interpretations of about equal probability.

These probabilities are: 1. That ether is *not acting primarily* on respiration and affects irritability in some other manner; or 2. That it is *acting primarily* on the respiratory process and affects both aerobic and anaerobic respiration in the same manner.

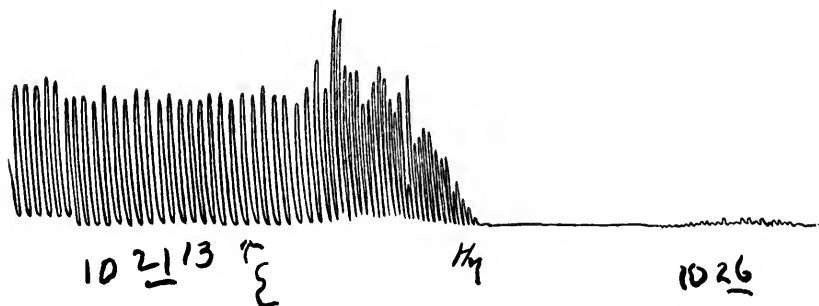


FIG. 2. Primary stimulation by the ether. Ether on ganglion at *E*. Rate amplitude increase. At *Hy*, the ether replaced by hydrogen. Read from left to right.

In deciding between these alternatives the facts may be recalled: that ether and other anesthetics suppress both aerobic and anaerobic respiration (4); that they suppress the organic synthesis of the cell, shown in growth, the formation of glycogen, starch and other substances (5); that for all aerobic tissues deprivation of oxygen causes some of the same series of changes as anesthetization, i.e., primary stimulation, depression, and after-stimulation (6) and similar chemical and physical changes; that the action of hydrocyanic acid, a substance which has a very intimate relation to respiration, is almost identical in all fundamental particulars with the anesthetic action, except that some cells permit of a complete suppression of their respiration by cyanide without per-

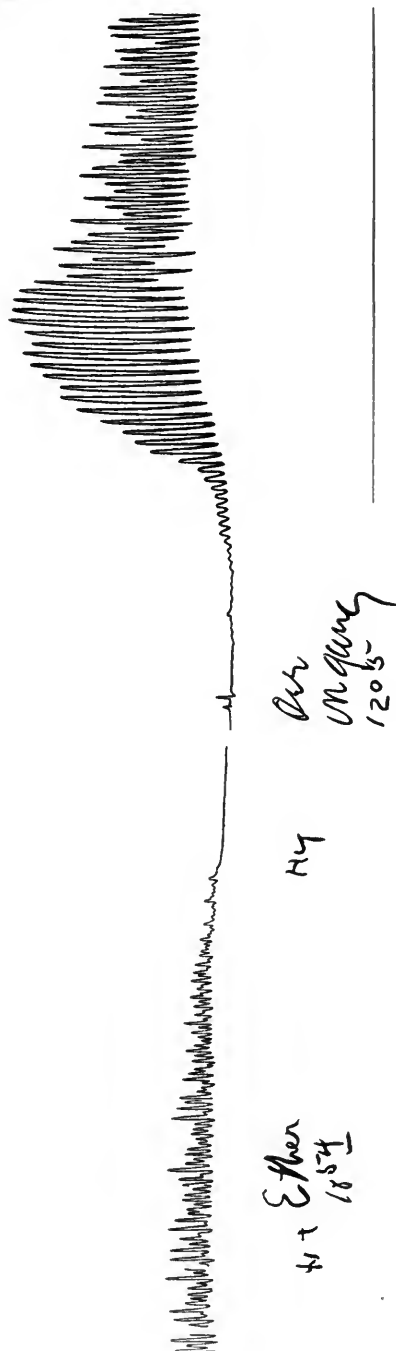


FIG. 3. Showing recovery in air, but not in hydrogen. The ganglion had been in hydrogen about one hour and previously anesthetized. At 11:54 the hydrogen was replaced by hydrogen and ether. At *Hy.* 11:56 the ether was replaced by hydrogen. No recovery occurred while the hydrogen was continued and at 12:05 air was substituted for hydrogen. Strong after-stimulation shown. Time marker: 1 minute intervals. Read from left to right.

manent harm, whereas complete suppression of respiration by ether is fatal (7). It may also be recalled that dividing sea-urchin eggs are especially sensitive to ether, acids, hydrocyanic acid and deprivation of oxygen at the same period of division, and are not so sensitive to any of them at other periods (8).

These facts appear to me to speak strongly in favor of the second of the alternatives. Dr. Carlson (3) has argued that since cyanides suppress the activity of the ganglion, and deprivation of oxygen does not, the cyanides cannot be acting primarily on respiration. But if cyanides acted in the same manner as indeed they seem to act, (9) on both aerobic and anaerobic respiration this argument would have no force.

The facts are that cyanides and the anesthetics check all of the fundamental phenomena of irritability. The remarkable chemical activity of the cyanides due presumably to the bivalent carbon they contain and their power of forming addition compounds which are more or less easily dissociable (10), and of checking oxidations both in and out of the cell (11) has led to the generally accepted conclusion that they act by means of their free chemical valencies, by chemically combining with the elements essential for irritability. We have no facts at present which indicate that the anesthetics effect protoplasm in any other than a chemical way, except the anesthetic efficiency of such substances as ether, and gasoline, which are chemically rather inert. Unfortunately we have no good means of measuring quantitatively the amount of dissociation and of chemical activity of organic compounds, so that it is impossible to compare accurately for each substance its chemical reactivity and anesthetic power, in a manner similar to the comparison of anesthetic action and the coefficient of fat solubility, made by Overton (12) and Meyer (13), but the indications are that such a comparison of chemical power would clear up many of the apparent anomalies of the fat-solvent anesthetic comparison *i.e.*, nitrous oxide (14). I tried some time ago to show that there is also a general parallelism (14) between the chemical reactivity of some of them and their anesthetic power, so that this again may be interpreted in favor of their acting chemically rather than physically.

While the possibility still remains that the anesthetics may be affecting respiration secondarily, by producing, or checking chemical interactions through their power of altering the physical structure of the protoplasm, the evidence appears to me to point more and more steadily to the conception that irritability is itself primarily a chemical phenomenon, involving the opening up or saturating of some of the valencies of either carbon, nitrogen, or oxygen, or some other atoms in the protoplasmic complex by means of which oxidations, syntheses, respiration, conduction and the other phenomena of irritability are brought to pass or inhibited, and that the physical changes are secondary to this chemical change. If this be the nature of the irritable process, then the interpretation becomes more probable that the anesthetics are effecting irritability by a direct union with these opening valencies, rather than by acting in a physical way primarily on the lipoids.

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PHARMACOLOGICAL STUDIES ON THE PHOSPHATIDS

1. METHODS FOR THE STUDY OF THEIR COMBINATIONS WITH DRUGS AND OTHER SUBSTANCES

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The study of the reaction of the cell to chemical substances has so far been principally directed to observations on such variations in physiological activity as are concerned with changes in irritability or functional activity. Our present knowledge of pharmacological facts and their application are largely built up on this physiological basis. The study of the more fundamental changes brought about in the chemical constituents of the cell, of which the physico-physiological changes are but an outward expression, has received less attention, largely due to our ignorance of the chemistry of the cell. Two of the most important contributions to Pharmacology: Overton and Meyer's "Theory of Narcosis" and Mathews' "Theory of Salt Action" are incomplete on this account. Thus Overton and Meyer's theory, although it establishes a very striking parallelism between fat solubility and narcotic action, is not based on any experimental evidence as to the action of the anaesthetics on the phosphatids, which are supposed to be the principle factors concerned. The work of Mathews, although it establishes a very definite parallelism between the solution tension of the ions of the salts and their toxicity to lower forms of life, does not give us any experimental basis as to the mechanism which enables potassium to exist in a higher proportion to sodium in the tissues than in the serum.

The recent extension of Ehrlich's theories into this field, with the introduction of the term chemo-receptor, can also be of little

help, as it gives us no definite notion as to the chemical nature of these receptors, nor of the nature of their specificity with regard to the action of drugs. As long as biologists hold to the notion of the giant molecule of Pflüger, not much progress can be made. Such inadequate theories as Loew's "System der Giftwirkungen" should serve as a warning. It is not difficult to recognize in Ehrlich's chemo-receptors, Loew's aldehyde groups put in a more fashionable garb. In observing such elaborations of Ehrlich's theories a chemist cannot help being reminded of the history of phlogiston. Starting at first as a useful tool, it became finally so elaborate and involved, that it was crushed by its own bulk and gave way to simpler conceptions based on a better knowledge of the fundamental facts.

In order to lay a foundation for the study, from the chemical point of view, of the changes which take place in the cell as the result of its interaction with chemical substances, the investigation of the chemistry of the nervous system was begun in this laboratory some years ago. The nervous system was selected not only because it is the tissue on which the action of drugs has been most studied, but also because it is very well suited for the study of fundamental cell problems on account of its high degree of differentiation, the ease with which it can be freed from the larger blood vessels and its very constant chemical composition. These chemical studies have led up to the conception, as has already been pointed out in a previous communication,¹ that we have to deal in a living cell with essentially two sets of factors.

1. The chemical activities which go on in watery solutions and involve the interaction of non-colloidal molecules of relatively small molecular weight.
2. The control exercised over these chemical activities by the larger or colloidal aggregates. These colloidal aggregates are not only the source of the differentiated anatomical units that appear to the eye as morphological structure, but undoubtedly are intimately concerned with the specific affinities of the cell.

¹ Koch, W., *The Journal of the American Chem. Soc.* 1909, xxxi, 1330.

Thus the embryonic neuron, with little anatomical differentiation in its cytoplasm, with a high water content and a large proportion of smaller, water-soluble, non-colloidal molecules, increases its colloidal material both relatively and absolutely as it differentiates and acquires its characteristic morphological structures such as the dendrites, axon and medullated sheath.

The problem of the relation of chemical substances (drugs, etc.) to such a cell as the neuron for instance and its activities has then to consider two sets of factors.

1. The factors which are involved in the selection and transmission of substances through the membranes of the cell into the body of the cell itself, factors among which the colloids are probably the most important.

2. The factors concerned with the changes brought about in the chemical or metabolic activities within the cell by the chemical substance after entrance has been gained.

In the following communications we will concern ourselves principally with the first set of factors; namely, the rôle played by the colloids, especially the phosphatids, in the entrance of chemical substances into a given cell.

The phosphatids, especially those from the nervous system, are particularly suitable, because they can be easily prepared in quantity and by means of solvents roughly divided into groups without altering too much their physical properties. The groups principally made use of in these studies were the following:

The lecithins: soluble in cold alcohol and ether, insoluble in acetone.

The kephalins: soluble in ether, insoluble in alcohol, hot or cold.

The lecithins proved more useful than the kephalins, which appeared to bear a much less important relationship to drugs, as will become apparent later.

On the assumption that one of the essential factors in the entrance of a substance into a given cell depends upon the power of that substance to combine with one of the colloids of that cell, the relationship of a large number of such substances to lecithin and kephalin were studied. In order to determine whether or

not a given substance has combined with a colloid, such as for instance lecithin, four principal methods are available, some of which have already been used by other investigators. They are as follows:

1. *The analytical method.* Concerns the study of the substance combined with the phosphatid, as it is isolated from the tissue. The phosphatid is put into watery emulsion, precipitated with an acid and the clear filtrate examined for non-colloidal water-soluble substances. This method has proven of great value in the study of the different ratios in which sodium and potassium occur in different phosphatids. The results in detail will be given later.

2. *The physical method.* Concerns the study of the physical properties such as the viscosity of a colloid. The viscosity of an emulsion of a given colloid is determined alone and with the addition of the substance to be tested. If there is a change in viscosity a chemical combination is supposed to have taken place. This method was used by Handowsky,² but was considered too laborious for our purposes.

3. *The physico-chemical method (a).* This method has been largely used in this laboratory³ and found to be very simple and at the same time sensitive. The principle consists in the estimation of the state of colloidal aggregation or the relative size of the particles of a given phosphatid by the measurement of its sensitivity to precipitation by standard calcium chloride solution. The calcium chloride brings about in some way, probably by the chemical combination with the colloid, an increase in the size of the colloidal particles which finally leads to a flocking out. If the colloidal solution is made up of particles of fairly uniform size this end point can be determined with considerable accuracy. Thus at the point where the flocking out takes place the tube in which the colloidal solution is still stable and the one in which the colloid flocks out completely should not differ in calcium content by more than 0.0001 molecular calcium chloride or 0.04 mg. of calcium in 10 cc. of solution.

² Handowsky, H. Biochem Zeitschrift, 1910, xxv, 510.

³ Koch, W. Zeitschrift für physiol. Chem., 1909, lxi, 432.

If now we add to a lecithin emulsion, standardized with this degree of accuracy, the substance to be tested, for instance bile salts, the amount of calcium required to precipitate is increased. Thus a lecithin emulsion with the precipitation limit at 0.0020 molecular calcium chloride (end point determined within 0.0001 molecular calcium chloride or accuracy of 5 per cent) requires in the presence of 0.01 per cent bile salts a concentration of 0.0032 molecular calcium chloride for precipitation (increase of 60 per cent with relative accuracy of 5 per cent).

The interpretation of this phenomenon is as follows: On account of the chemical affinity between the bile salts and the lecithin a combination has taken place by which the surface tension or attraction of the molecules of the colloidal particles for one another has decreased and as a result their relative state of aggregation has been decreased so that more calcium is required to bring them again to the flocking out or precipitation point. The possibility must also be considered that the bile salts may have brought about this change by altering the relations of the colloidal particles and the surrounding medium, namely, the watery solution. Subsequent work has, however, made the first supposition more probable and it is fairly certain that we are enabled by this means to test the affinity of a large number of substances for lecithin. Whenever a substance added to a lecithin emulsion decreases the size of the particles so as to require larger amounts of calcium for precipitation, we may conclude that there is a certain amount of chemical affinity between this substance and lecithin.

Some substances, as for instance the calcium chloride used in these tests, increase the size of the colloidal aggregates and this phenomenon may also be interpreted as evidence of chemical combination of another kind however. In this case, namely, the reacting substance must add energy to the system, which energy is expended in increasing the attractions of the molecules making up the colloidal particles for one another and thus increasing the size of the colloidal aggregate, sometimes up to the precipitation point. Only substances possessing a low solution tension such as the heavy metals, the acids or substances dissociating so as to

liberate hydrogen ions (carbon dioxide in watery solution) produce a change in this direction.

If the phosphatid consists of a mixture of several colloids, having a different state of aggregation, the end point will not be sharp. This difficulty was sometimes encountered in the case of kephalin. The details of technique and results will be given under (3 and 4).

(b) Method of Bing.⁴ The substance to be tested is evaporated in alcohol solution of the phosphatid and the physical and chemical properties of the resulting residue studied. A change in solubility is interpreted as indicating chemical combination. This method lacks refinement and subjects the substances to be tested to rather unphysiological conditions. It was not made use of in these studies.

4. *The physiological method.* The method depends on the insolubility of the substance to be tested in water and its solubility in the presence of the phosphatid. It is best illustrated by an example. Strychnin (free alkaloid) is shaken with water for an hour, the solution filtered and 0.3 cc. injected into a 40 g. frog. Tetanus occurs in about 30 to 40 minutes.

Strychnin (free alkaloid) is shaken with a 0.3 per cent watery emulsion of lecithin for one hour. The solution filtered and 0.3 cc. injected in a 40 g. frog. Tetanus occurs in 2 to 3 minutes. The conclusion is that strychnin has combined with lecithin. This is confirmed by the method given under 3a.

Lecithin emulsion alone requires 0.0022 molecular CaCl_2 .

Lecithin emulsion plus strychnin requires 0.0028 molecular CaCl_2 .

These differences are well beyond the experimental error.

The details of technique and results will be given under (5).

In the following pages the results obtained with the above methods will be given. They indicate that a large number of substances have the power to combine with the phosphatids more or less firmly. In fact the number is so great that those substances which were found not to show evidence of combination promise to be rather more interesting.

⁴ Bing, Skandinavisches Arch., 1901, xi, 166.

2. THE RELATION OF THE PHOSPHATIDS TO THE SODIUM AND POTASSIUM OF THE NEURON

W. KOCH AND F. H. PIKE

The fact that the phosphatids like all colloids can only with difficulty be freed from inorganic constituents has occasionally received notice. Thus Thudichum¹ and more recently Fränkel² mention the presence of potassium in kephalin. Rosenheim³ estimates potassium in protagon and Burow⁴ mentions the presence of iron in phosphatids from the spleen. In a study by one of us⁵ on the precipitation of lecithin by salts, the possibility of combinations in molecular proportions was also suggested. No systematic investigations are however on record, which is especially surprising when we consider, for instance, the importance which Macdonald⁶ attaches to the presence of potassium in the nerve fibres. Moore⁷ assumes that the potassium is combined with the proteins and that the combination is in the nature of an adsorption of some salt of potassium by the colloidal complex. The idea that an ion-like potassium may directly combine with the colloid, so that the colloidal complex acts as the anion has never been clearly stated except by Mathews.⁸

In the following pages evidence will be given not only that potassium and sodium of the tissues are combined with the phosphatids,

¹ Thudichum: *Die Chemische Konstitution des Gehirns des Menschen und der Tiere*. 1901, F. Piezcher. Tübingen, pp. 17, 85, 131.

² Fränkel S.: *Wiener medicinsche Wochenschrift*. 1909, No. 47.

³ Rosenheim, O., and Tebb, Christine M.: *Quarterly Journal of Experimental Physiology*. 1909, ii, 324.

⁴ Burow, R.: *Biochemische Zeitschrift*. 1910, xxv, 165.

⁵ Koch, W.: *Zeitschrift für physiologische Chemie*. 1909, lxxiii, 432.

⁶ Macdonald: *Proceedings Royal Society, B*. 1905. lxxvi, 322.

⁷ Moore, B.: *Further Advances in Physiology*. Edited by L. Hill. 1909, i, 17.

⁸ Mathews, A. P.: *Biological Studies by the Pupils of W. T. Sedgwick*, Boston, 1901.

rather than with the proteins, but also that the combination is in the nature of an ion-colloid combination. A further interest attaches to the results from the fact that in these combinations specific affinities appear to play a rôle, as it was found that in kephalin the potassium greatly exceeds the sodium, while in lecithin the reverse is true.

ESTIMATION OF SODIUM AND POTASSIUM IN BRAIN AND CORPUS CALLOSUM

The method of procedure was to thoroughly extract the tissue with alcohol and ether as outlined in "Methods for the Quantitative Chemical Analysis of Animal Tissues."⁹ The alcohol and ether-soluble portion was evaporated to dryness, emulsified with water and the lipoids including the phosphatids precipitated with nitric acid. The filtrate was then used for the estimation of sodium, potassium, calcium, magnesium, chlorides, sulphates and phosphates. Calcium and magnesium sulphates were only found in traces in this fraction. In the case of the corpus callosum, the principal tissue studied in this investigation, the ash in this fraction represented about 80 per cent of the total ash and contained 95 per cent of the total potassium. Unless the heating employed in the extraction liberated the salts from the proteins this may be taken as evidence that the potassium at least is not in combination with the proteins.

TABLE I

	PER CENT OF DRY TISSUE IN						K	PER CENT OF TOTAL IN ORGANIC COMBINATION.	RATIO OF K:Na IN MOLECULAR PROPORTION.
	K	Na	Ca,Mg	Cl	PO ₄	SO ₄			
Whole brain.....	0.95	0.87	trace	0.72	0.5	0.03	0.54	57	1:1.55
Corpus callosum	0.95	0.33	0.01	0.49	0.3	0.03	0.70	73	1:0.60

*In calculating this figure the assumption was made that all the sodium was combined with the chlorides for which it more than accounts and that the phosphates found were represented by the compound K_2HPO_4 . Whatever fallacies are involved in this assumption will increase rather than decrease the amount of potassium not accounted for by the anions found.

⁹ Koch, W.: The Journal of the American Chemical Society. 1909, xxxi, 1329.

In human blood serum the ratio of potassium to sodium is about 1:18 (Hammarsten). The above table indicates that the much greater proportion of potassium in the brain must be due to the presence of potassium in some other combination besides chlorides, phosphates, or sulphates. Approximately 57 per cent remains to be accounted for. In the corpus callosum this proportion is still higher. In other words we are dealing here with the same problem which was proven so difficult in the study of other tissues. It is necessary to look for other possible compounds of potassium. From the fact that practically all the potassium goes into the alcohol solution it seemed most promising to look for such compounds among the lipoids rather than among the proteins. The following table gives the results of this search. The preparation of lecithin and kephalin have been already described.¹⁰ The preparation of the sulfatid will be described at a later date.

TABLE II

	PER CENT OF DRY SUBSTANCE IN						K	PER CENT OF TOTAL IN OR- GANS COMBI- NATION	RATIO OF K:Na IN MOLECULAR PROPORTION
	K	Na	CaMg	Cl	PO ₄	SO ₄			
Lecithin.....	0.34	0.80							1: 4.00
Kephalin.....	1.69	0.72	0.03	0.0	0.42	0.0	1.35	80	1: 0.70
Sulfatid.....	3.10	0.82		0.0		0.0	3.10		1: 0.45

Of interest in the above table is the fact that lecithin not only has a much lower content of sodium and potassium than kephalin, but also that the ratio is completely reversed in the two compounds. This observation was also confirmed in the case of lecithin prepared from eggs. The results point quite clearly to the fact that in kephalin and the sulfatid the potassium is in combination with the colloid. This gives us one possibility of accounting for the accumulation of potassium in a tissue. Potassium can dissociate from such a compound, but on account of the relative

¹⁰ Koch, W.: Zeitschrift für physiologische Chemie. 1902, xxxvi, 134.

immobility of the colloidal anion, cannot diffuse to any great extent without the production of very large differences of potential. The application of these findings to Macdonald's theory of the nerve impulse will be considered in another communication by one of us (F. H. P.). The lecithin and kephalin here analysed were the preparations used in the subsequent investigations.

The detailed investigation of the methods here outlined and their application to other tissues will be published later by C. C. Todd to whom we are indebted for a number of the analyses here recorded. For the estimation of potassium the method of Adie and Wood as modified by Bowser¹¹ was used.

SUMMARY

The greater concentration of potassium in the cells of a tissue as compared to the surrounding lymph spaces or serum, can be in part accounted for by the specific affinity for this element, of some of the phosphatids, especially kephalin.

¹¹ Bowser, L. T.: *The Journal of Industrial and Engineering Chemistry*. 1909, 794. Adie and Wood. *Journal of the Chemical Society*. lxxvii, 1076.

3. THE RELATION OF THE PHOSPHATIDS TO OVERTON AND MEYER'S THEORY OF NARCOSIS

W. KOCH AND F. C. McLEAN

The older theories of narcosis have been so well reviewed in Overton's¹ book as not to require further mention. Overton and Meyer's² hypothesis, arrived at independently, that the narcotic power of a substance is dependent on its distribution coefficient between oil and water is too well known to require detailed description. The more recent attempt of Traube³ to bring the property of a good many narcotics to produce changes of surface tension into relation with the phenomenon of narcosis is not sufficiently general. Thus the very striking relationships which Meyer discovered in the sulfonal series cannot be accounted for by any changes in surface tension. The work of Moore and Roaf⁴ on the action of chloroform on proteins need hardly be taken seriously, as they used concentrations which were much above the concentration of chloroform ever to be expected in the tissues during narcosis. The lack of regard for such quantitative relationships is a very common source of error in pharmacological investigations of this type.

The present investigation was undertaken with the purpose of establishing, if possible, an experimental basis for the suggestion implied in both Overton and Meyer's work, namely, that changes in the physical state of the phosphatids may account for the phenomenon of narcosis. To demonstrate this required the investigation of the following points:

¹ Overton: *Theorie der Narcose*.

² Meyer, H.: *Archiv. f. exper. Pharm. and Path.*, 1899, xlii, 109 and 119 and 1901, xlvi, 338 and 347.

³ Traube, J.: *Pflügers Archiv*. 1904, cv, 541 and 559, and 1908, cxxiii, 419.

⁴ Moore, B. and Roaf, H. E.: *Proc. Royal Soc. B.*, 1905, lxxvii, 90.

1. Can changes in the state of aggregation of the phosphatids, provided they are produced by anaesthetics and narcotics, be made to account for the phenomenon of narcosis?

2. Is there any evidence that the anaesthetics enter into combination with the phosphatids or produce changes in their state of aggregation?

The two chemical substances which produce the most marked changes in the state of aggregation of the phosphatids are acids and alkali, as has already been pointed out in a previous communication.⁵ Acids increase the surface tension and cause the formation of larger aggregates, thus decreasing the amount of calcium required to precipitate. Physiologically acids produce a condition of decreased irritability which may bear some relation to the phenomenon of narcosis. Alkalies decrease the surface tension and cause the formation of smaller aggregates, thus increasing the amount of calcium required to precipitate. Physiologically alkalies, if not used in too great concentration, have a tendency to produce a condition of increased irritability. From these considerations one might expect the anaesthetics and narcotics to resemble the acids in their action. In the following table a number of substances were arranged with regard to their ability to change the state of aggregation of a 0.15 per cent emulsion of brain lecithin, as determined by the amount of calcium chloride required to produce a precipitate:

TABLE I

INCREASE IN SIZE OF COLLOIDAL PARTICLES*	NO CHANGE	INCREASE IN SIZE OF COLLOIDAL PARTICLES†
Acids	Chloral	Alkalies
Alcohol	Hypnon	Chloroform
Triacetin	Acetone	Sulfonal
Paraldehyde	Ether (pure)	Ammonia
Ether (impure)		Bile Salts

*Except in the case of acids the amount of change with the substances of the first column is very slight and can just about be detected.

†The change produced by the substance of this column is very considerable (see 4) Koch and Williams.

⁵ Koch, W., *Zeitschrift für physiologische Chemie.*, 1909, lxi, 432.

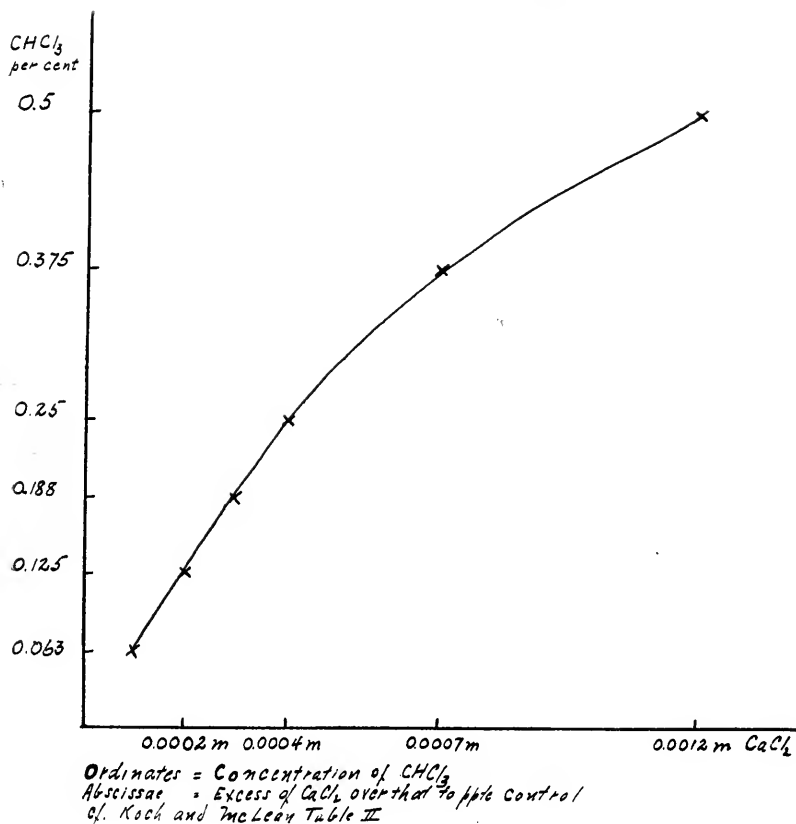
The absence of any relation between changes in physical state of aggregation and the power of the above substances to act as narcotics or anaesthetics is so plainly apparent, that it was not considered worth while to extend the above table. One point is, however, of considerable interest; namely, the difference between chloroform and pure ether, which produces no change. If we interpret the decrease in size of particles as evidence of one kind of chemical combination we have here an explanation of the slower rate at which chloroform is eliminated from the tissues and its consequent tendency to produce delayed poisoning. In order to make certain that the small amounts of chloroform which can be demonstrated to be actually present in the tissues during anaesthesia, are capable of producing this change, the action of chloroform was investigated more in detail.

Experiment: 100 cc. of a 0.3 per cent emulsion of brain lecithin was shaken for one hour with 1 gram of chloroform. The precipitation limit of this emulsion was then compared with a control (0.3 per cent) solution of lecithin. The chloroform solution required for precipitation a concentration of 0.0027 m. CaCl_2 , while the control required 0.0015 m. CaCl_2 , a difference of 1.2 cc. expressed in 0.01 m. The chloroform lecithin emulsion was then diluted with lecithin emulsion to determine the smallest quantity of chloroform which has any appreciable effect. The results are given in the following table and also plotted in the form of a curve:

TABLE II

CHLOROFORM	CaCl_2 to PPT.	CONTROL	EXCESS OF CaCl_2
<i>per cent.</i>	<i>m.</i>	<i>m.</i>	<i>m.</i>
0.5	0.0027	0.0015	0.0012
0.375	0.0022	0.0015	0.0007
0.25	0.0019	0.0015	0.0004
0.188	0.0018	0.0015	0.0003
0.125	0.0017	0.0015	0.0002
0.063	0.0016	0.0015	0.0001
0.000	0.0015	0.0015	0.0000

The lowest concentration of chloroform which produced a noticeable effect was 0.063 per cent, an amount which has actually



been found by several investigators to be present in tissues during chloroform narcosis.

Some experiments were tried with kephalin emulsions, and they yielded similar but less striking results.

SUMMARY

There is no evidence that anaesthetics or hypnotics produce changes in the state of aggregation of lecithin or kephalin, which are sufficiently consistent, to account for such a general phenomenon as narcosis. There is some evidence, however, that chloroform as distinguished from pure ether has the power to form a combination with lecithin, a phenomenon which may be brought into relation with its slow elimination and consequent tendency to produce delayed poisoning.

4. THE RELATION OF BRAIN PHOSPHATIDS TO TISSUE METABOLITES

W. KOCH AND A. W. WILLIAMS

The observation that the accumulation of potassium in the cell can be more satisfactorily accounted for by the fact that it is combined with kephalin than on the theory of any hypothetical semipermeable membrane ought to be capable of extension to the tissue metabolites. The tendency of a tissue metabolite on the one hand to accumulate in, and on the other hand to be eliminated from the cell in which it has been manufactured: its ability when eliminated from the first cell to enter into and accumulate in other cells or to be immediately excreted through the kidney, no doubt bears some relation to its power of combination with tissue colloids. Thus kreatin accumulates in the muscle to the extent of 0.4 per cent while urea is never found in any tissue except in minimal amounts. Ammonia is formed in the intestine but goes to the liver, there to enter into chemical transformations and be finally eliminated as urea. Adrenalin is manufactured in the suprarenal, but disappears from the blood so rapidly that it is difficult to detect its presence. All the above substances are sufficiently easily soluble in water, to expect, on the theory of osmosis, that they would be uniformly eliminated through the kidney. Specific chemical affinities very probably play a rôle and the present investigation was undertaken to determine to what extent the phosphatids possess the power of combining with products of tissue metabolism. Instead of the direct analytical method used for potassium, the calcium chloride method (3a) was employed in order to permit the examination of a larger number of substances.

Details of method: The outline of this method has already been given. A 0.3 per cent emulsion of lecithin is made by shaking the required amount of lecithin for several hours in a shaking machine

until it has all been emulsified. The substance to be tested is then shaken up with a part of this solution. Twice as much substance as is required for the final concentration must be used. To a series of test tubes $\frac{M}{100}$ calcium chloride solution is then added in increasing amount so that at least three or four of the tubes will show a precipitate in order to make sure of the end point. Water is then added to make the volume 5 cc. and then 5 cc. of the solution to be tested. The tubes are gently shaken and allowed to stand. After about twenty hours or better the next day the tubes are read.

The following will illustrate:

0.3 PER CENT LECITHIN, 0.04 BILE SALTS. ADD 5 CC. TO EACH TUBE			0.3 PER CENT LECITHIN, NO BILE SALTS. ADD 5 CC. TO EACH TUBE		
$\frac{M}{100}$ CaCl ₂	Water	Result after 20 hours	$\frac{M}{100}$ CaCl ₂	Water	Result after 20 hours
cc.	cc.		cc.	cc.	
3.3	1.7	—	1.5	3.5	—
3.4	1.6	—	1.6	3.4	—
3.5	1.5	—	1.7	3.3	—
3.6	1.4	—	1.8	3.2	—
3.7	1.3	—	1.9	3.1	—
3.8	1.2	+	2.0	3.0	+
3.9	1.1	+	2.1	2.9	+
4.0	1.0	+	2.2	2.8	+
4.1	0.9	+	2.3	2.7	+
4.2	0.8	+	2.4	2.6	+

— indicates no precipitate. + indicates a precipitate.

This makes the total volume 10 cc. in each tube and gives a final concentration of 0.15 per cent lecithin and 0.02 per cent bile salts. In this case then the presence of a concentration of 0.02 per cent bile salts so decreases the surface tension and consequently the size of the colloidal particles of lecithin as to require 1.8 cc. more of $\frac{M}{100}$ CaCl₂ to produce a precipitate. In running a series with different concentrations of the substance to be tested it is more convenient and accurate to make up the strongest concentration first and then make up the other concentration by diluting with the required amount of lecithin emulsion or control solution.

Sources of error: The *accuracy of the end point* is usually very satisfactory with a good preparation of lecithin. A difference of 0.1 cc. of $\frac{M}{100}$ CaCl_2 in 10 cc. corresponding to 0.04 mg. CaCl_2 is readily detected. With kephalin the end point is not nearly so sensitive. A control of the end point of a given preparation of lecithin must be made every day or rather with every series. In work with colloids it is impossible to make too many control experiments. In the course of several weeks it was found that the preparation of lecithin which at first precipitated with 0.0020 m. CaCl_2 required 0.0028 m. CaCl_2 . The reason for this is not quite clear. It cannot be due to formation of acids by hydrolysis of lecithin as that would shift the end point in the opposite direction. As this change can be somewhat controlled by keeping the preparation in vacuum over calcium chloride or in an atmosphere of carbon dioxide it is not unlikely that it is associated with oxydation. The source of error due to this variation was eliminated by carrying on a control experiment every day.

The effect of *temperature* on the end point appeared to be of some significance as several times when the tubes were left over night in a very warm room the results were contradictory. Experiment directed to establish this point by carrying on an experiment in the ice box and in a very warm room did not, however, confirm this suspicion. Heating of the lecithin emulsion to near the boiling point and then allowing to cool did not materially affect the end point.

The substances studied may be divided into three classes according to the results obtained.

1. *Substances which not only combine with the lecithin, but so alter the state of aggregation of the lecithin as to account for alterations in the permeability of the cell.* Sodium chloride, ammonia and bile salts.

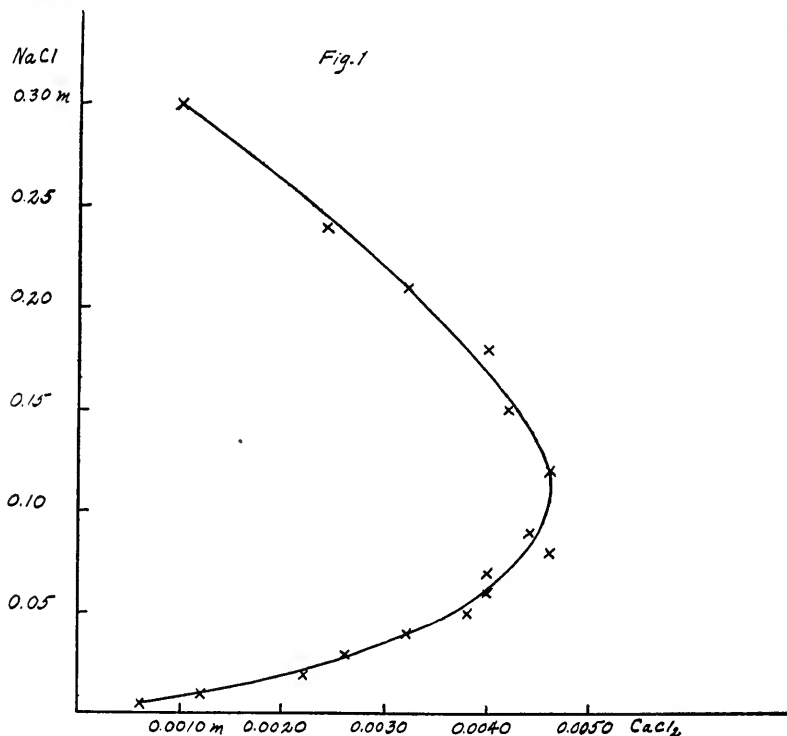
Sodium Chloride is of course not to be classed among tissue metabolites, but was here considered on account of its influence on the conditions under which tissue metabolites act. The results are given in the following table and plotted in the accompanying curve (Fig 1):

TABLE I

NaCl	CaCl ₂ to PPT. LECITHIN		CONTROL	EXCESS CaCl ₂	
m.	m.	m.	m.	m.	m.
0.005	0.0058		0.0052	0.0006	
0.01	0.0062 to 0.0064		0.0052	0.0010 to 0.0012	
0.02	0.0072 to 0.0074		0.0052	0.0020 to 0.0022	
0.03	0.0076 to 0.0078		0.0052	0.0024 to 0.0026	
0.04	0.0080 to 0.0084		0.0052	0.0028 to 0.0032	
0.05	0.0084 to 0.0090		0.0052	0.0032 to 0.0038	
0.06	0.0086 to (0.0092)*		0.0052	0.0034 to (0.0040)	
0.07	0.0086 to (0.0092)		0.0052	0.0034 to (0.0040)	
0.08	0.0090 to (0.0098)		0.0052	0.0038 to (0.0046)	
0.09	0.0092		0.0048	0.0044	
0.12	0.0094		0.0048	0.0046	
0.15	0.0090		0.0048	0.0042	
0.18	0.0088		0.0048	0.0040	
0.21	0.0076 to 0.0080		0.0048	0.0028 to 0.0032	
0.24	0.0068 to 0.0072		0.0048	0.0020 to 0.0024	
0.30	0.0052 to 0.0058		0.0048	0.0004 to 0.0010	

* Figures in parentheses are estimated.

In the second column the first set of figures denotes partial precipitation, the second set complete precipitation.



This series was run with a different sample of lecithin from that of Tables II and III, hence the difference in controls.

In the interpretation of the sodium chloride curve we must consider that the sodium (Na^+) and chlorine (Cl^-) ions act more or less independent of one another. Thus as we begin to increase the amount of sodium chloride, there is a decrease in the state of aggregation of the lecithin, with a consequent increase in the amount of calcium chloride required to precipitate. This change is due to the chlorine ion, which antagonizes not only the precipitating action of the calcium, but also that of the sodium.

The Cl^- ion has this property in common with Br^- , I^- and OH^- the hydroxyl being the most powerful and the chlorine the least. The reaction is probably in the nature of a loose chemical combination of the chlorine ion with the lecithin, somewhat on the order of the phenomena studied by Moore and Bigland¹ with proteins.

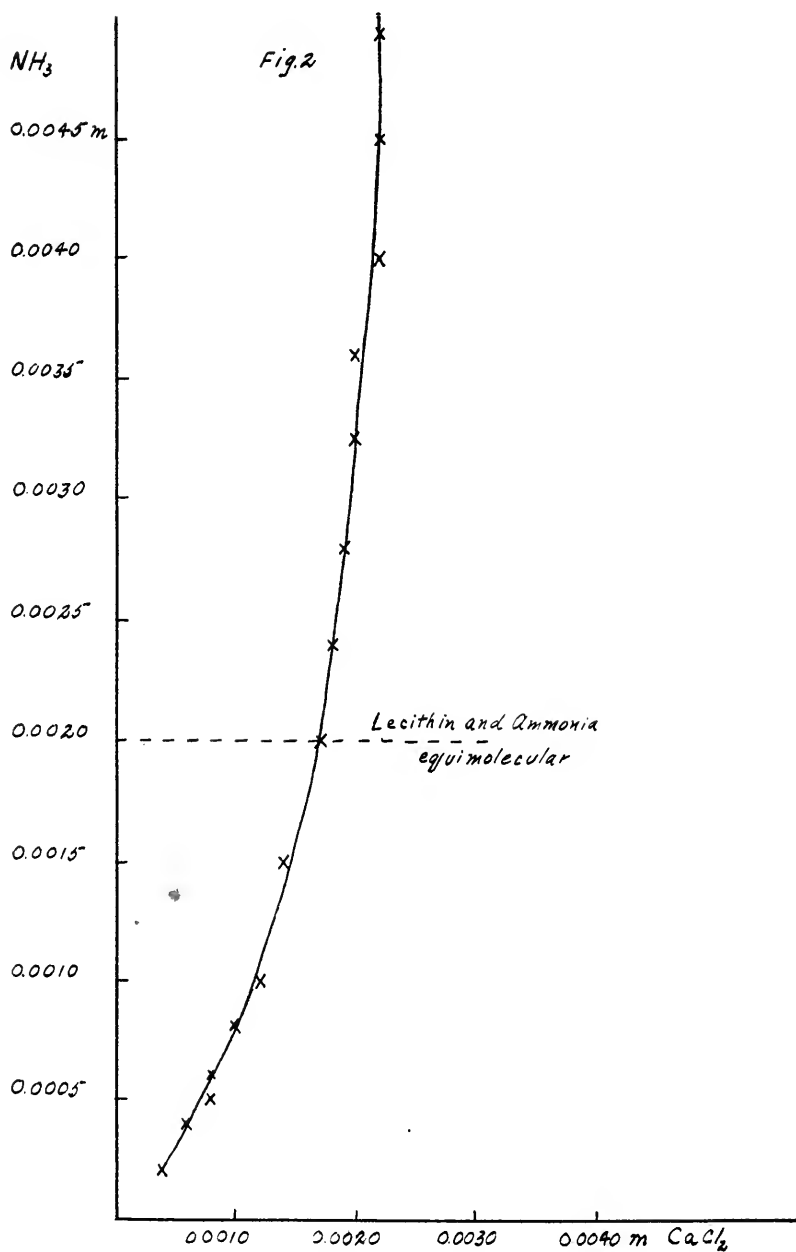
When the concentration of the sodium chloride reaches that of a so-called physiological salt solution (0.12 molecular), the action of the chlorine is no longer sufficient to counteract the sodium and calcium and they now begin to combine their action, so that precipitation becomes easier and easier until finally sodium itself precipitates without the addition of calcium.

These observations are capable of application to the phenomenon of chloride retention by the tissues. If we change the initial state of aggregation of the lecithin by a third substance (bile salts) the power of the chlorine ion to combine with lecithin can be increased above that of a physiological salt solution. The detailed working out of this suggestion will be taken up at an early date.

Ammonia is a true tissue metabolite and its functional significance has already been referred to in a previous article.² The results are given in the following table and plotted in the accompanying curve (Fig. 2).

¹Moore, B., and Bigland, I. D.: *Biochemical Journal*, 1910, v, 32.

²Koch, W.: *Zeitschrift für physiolog. Chem.*, 1909, lxxiii, 432.



Ordinates = Concentration of Ammonia
 Abscissae = Excess of CaCl_2 over that to ppt. Control
 Cf. Koch and Williams, Table II

TABLE II

NH ₄ OH	CaCl ₂ TO PPTE. LECITHIN	CONTROL	EXCESS CaCl ₂
m.	m.	m.	m.
0.0002	0.0032	0.0028	0.0004
0.0004	0.0034	0.0028	0.0006
0.0005	0.0036	0.0028	0.0008
0.0006	0.0036	0.0028	0.0008
0.0008	0.0038	0.0028	0.0010
0.0010	0.0040	0.0028	0.0012
0.0015	0.0042	0.0028	0.0014
0.0020	0.0045	0.0028	0.0017
0.0024	0.0046	0.0028	0.0018
0.0028	0.0047	0.0028	0.0019
0.0032	0.0048	0.0028	0.0020
0.0036	0.0048	0.0028	0.0020
0.0040	0.0050	0.0028	0.0022
0.0045	0.0050	0.0028	0.0022
0.0050	0.0050	0.0028	0.0022

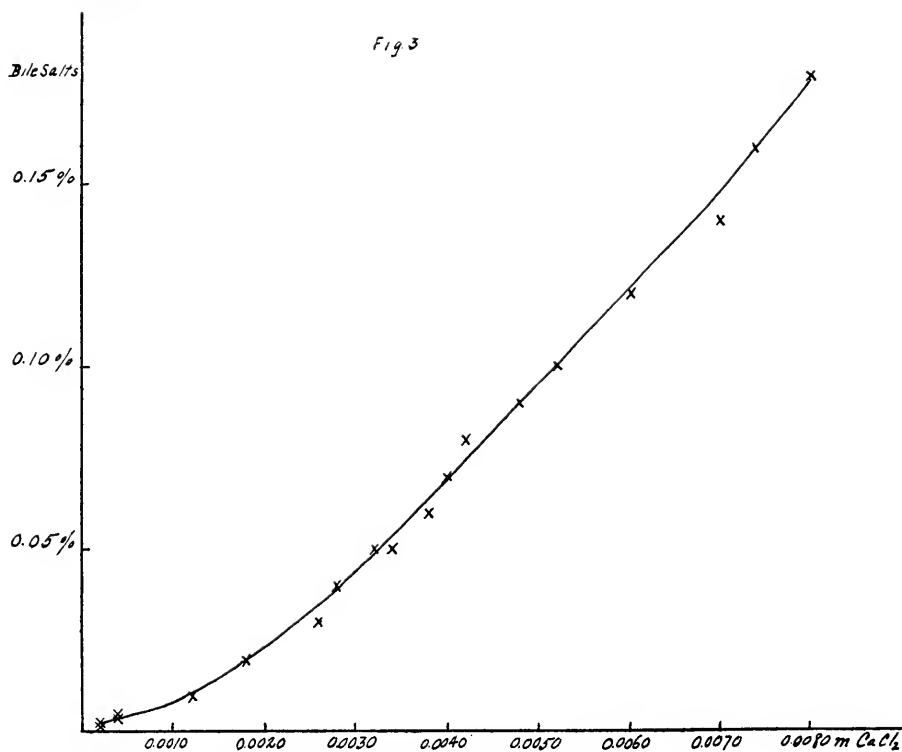
It will be seen that the ammonia curve differs very essentially from the sodium chloride curve. If we consider that a 0.15 per cent lecithin emulsion represents about 0.002 m. lecithin it will be observed that the effect of ammonia tends to become constant after molecular proportions between it and the lecithin have been passed. In other words a further increase in ammonia does not materially alter the amount of calcium chloride required to precipitate. The curve has no sharp break but rather a gradual slope which is, however, quite in harmony with similar observation on colloidal material. To expect the law of definite proportions to hold with colloids as it does with simple compounds can only lead to such errors as we are already familiar with in immuno chemistry.

An attempt to repeat these studies with kephalin revealed the interesting fact that the end point of a kephalin emulsion is not nearly so sensitive to ammonia. Here again we meet with the phenomenon of specific differences of tissue phosphatids in their relation towards chemical substances.

The *Bile Salts* may be regarded as having a more important relation to the animal economy than as mere excretory products. No attempt was made to study them as individuals. The results are given in the following table and plotted in the accompanying curve (Fig. 3):

TABLE III

BILE SALTS	CaCl ₂ TO PPT. LECITHIN	CONTROL	EXCESS CaCl ₂
<i>per cent.</i>	<i>m.</i>	<i>m.</i>	<i>m.</i>
0.001	0.0022	0.0020	0.0002
0.002	0.0022	0.0020	0.0002
0.003	0.0024	0.0020	0.0004
0.005	0.0024	0.0020	0.0004
0.010	0.0032	0.0020	0.0012
0.020	0.0038	0.0020	0.0018
0.030	0.0046	0.0020	0.0026
0.040	0.0048	0.0020	0.0028
0.050	0.0052	0.0020	0.0032
0.060	0.0058	0.0020	0.0038
0.070	0.0060	0.0020	0.0040
0.080	0.0062	0.0020	0.0042
0.090	0.0068	0.0020	0.0048
0.100	0.0072	0.0020	0.0052
0.120	0.0080	0.0020	0.0060
0.140	0.0090	0.0020	0.0070
0.160	0.0096	0.0020	0.0076
0.180	0.0100	0.0020	0.0080



The nature of this curve again differs from the other two and has a tendency to approach a straight line. The remarkable point about both ammonia and bile salts is the very small amount which produces a noteworthy effect on lecithin. Ammonia can be detected in a dilution of 1:500,000 and bile salts in a dilution of 1:100,000. Substances which can so exquisitely alter the physical state of colloids, must play an important rôle in the regulatory activities of the body and the relation of the tissues to one another.

2. *Substances which combine with lecithin but do not appreciably alter the state of aggregation, in the concentration in which they may be expected to exist in the tissues.* They were usually studied in concentration from 1:500 and 1:1000. The results are recorded in the following table:

TABLE IV

METABOLITE	CONCENT. CaCl_2 TO PPT. LEC.		CONTROL	DIFFERENCE OF CaCl_2
	per cent.	m.	m.	m.
Inosit.....	0.2	0.0022	0.0022	0
Inosit.....	0.2	0.0022	0.0021	+ 0.0001
Inosit.....	0.2	0.0024	0.0022	+ 0.0002
Hypoxanthin.....	0.2	0.0022	0.0021	+ 0.0001
Hypoxanthin.....	0.2	0.0023	0.0021	+ 0.0002
Hypoxanthin.....	<0.2	0.0024	0.0022	+ 0.0002
Kreatin.....	0.2	0.0023	0.0021	+ 0.0002
Kreatin.....	0.2	>0.0024	0.0022	+>0.0002
Kreatin.....	1.0	>0.0036	0.0026	+>0.0010
Kreatinin.....	0.2	0.0024	0.0021	+ 0.0003
Kreatinin.....	0.2	>0.0024	0.0022	+>0.0002
Kreatinin.....	1.0	0.0036	0.0026	+ 0.0010
Adrenalin.....	<0.1	>0.0026	0.0022	+>0.0004
Adrenalin.....	<0.1	0.0036	0.0026	+ 0.0010
Urea.....	0.2	0.0021	0.0021	0
Urea.....	0.2	0.0023	0.0022	+ 0.0001
Urea.....	1.0	0.0026	0.0026	0
Triäthylaminchlorhydrate.	0.1	>0.0024	0.0022	+>0.0002
Ammonium carbonate.....	0.001	0.0040	0.0028	+ 0.0012
CO_2	$\frac{1}{2}$ Sat'd	0.0021	0.0021	0
CO_2	Sat'd	0.0021	0.0021	- 0.0001
Lactic acid.....	0.001	0.0024	0.0028	- 0.0004

< in column two indicates that the concentration was not obtained because of insolubility. In two of the three hypoxanthin experiments it was dissolved in boiling water.

In this series each test tube varies by 0.0001 m. CaCl_2 , except in the case of adrenalin, 0.0002 m.

The results indicate that hypoxanthin, kreatin, kreatinin, adrenalin, triaethylamin (used in place of cholin which was not available) and ammonium salts combined with lecithin. Inosit is doubtful and urea does not combine. Carbon dioxide and lactic acid like all acids render lecithin more sensitive. That adrenalin forms a salt with lecithin is further confirmed by the green color given with a drop of ferric chloride which is characteristic of adrenalin salts as distinguished from the free base.

3. *Substances which may be regarded as of food value to the tissues* were next tried. The results are given in the following table.

TABLE V

TISSUE FOODS, ETC.	CONCENT. CaCl_2 TO P.P.T.E. LEC.		CONTROL	DIFFERENCE OF CaCl_2
	per cent.	m.	m.	m.
Aspartic acid.....	0.1	<0.0024	0.0028	->0.0004
Glutaminic acid.....	0.1	0.0027*	0.0028	- 0.0001
Tyrosin.....	<0.1	<0.0028	0.0028	0
Histidin.....	0.1	>0.0032	0.0028	+>0.0004
Cystin.....	<0.1	0.0030	0.0028	+ 0.0002
Leucin.....	0.1	0.0030	0.0028	+ 0.0002
Alanin.....	0.1	0.0034	0.0028	+ 0.0006
Glycocol.....	0.1	0.0032	0.0028	+ 0.0004
Glucose.....	0.1	0.0030	0.0028	+ 0.0002
Glucose.....	0.2	0.0030	0.0028	+ 0.0002

* Interpolated.

In this series each tube varied by 0.0002 m. in CaCl_2 .

The monobasic amino acids with the exception of tyrosin combine and act like basic substances as also does glucose. The dibasic acids behave like acids in general. The results indicate that there is some power of combination.

4. The extension of these observations to *substances which have a characteristic physiological action* seemed of interest. Some preliminary experiments are recorded in the next table.

Some interesting differences are to be observed and the extension of these observations, especially to phosphatids from other tissues seems promising. Some experiments with heart phosphat-

TABLE VI

DRUGS	CONCENT. CaCl ₂ TO PPT. LEC.	CONTROL	DIFFERENCE OF CaCl ₂
	<i>per cent.</i>	<i>m.</i>	<i>m.</i>
Digitalin.....	0.1	0.0024	0.0022 + 0.0002
Digitalin.....	0.1	0.0022	0.0021 + 0.0001
Saponin.....	0.1	0.0026	0.0026 0
Adrenalin.....	<0.1	>0.0026	0.0022 + >0.0004
Adrenalin.....	<0.1	0.0036	0.0026 + 0.0010
Strychnin.....	<0.1	0.0028	0.0022 + 0.0006
Strychnin.....	<0.1	0.0038	0.0028 + 0.0010
Strophanthin.....	0.1	0.0034	0.0028 + 0.0006
Caffein.....	0.2	0.0028	0.0028 0
Theobromin.....	<0.2	0.0028	0.0028 0

ids were begun by C. G. McArthur and yielded interesting results which will be published when they have been fully investigated.

SUMMARY

The observations recorded in this paper indicate:

1. That the changes in state of aggregation of lecithin produced by sodium chloride are the result of the independent action of the sodium and chlorine ions, whose effects are in opposite directions. Below the concentration of a physiological salt solution (0.12 molecular) the action of the chlorine ion, which decreases the state of aggregation of the lecithin, predominates. Above the concentration of a physiological salt solution, the action of the sodium ion, which tends to increase the state of aggregation of lecithin, comes more and more into prominence.

It has been suggested that when the phenomenon of chloride retention occurs, some change has taken place in the state of aggregation of the cell lipoids, which allows this action of the chlorine ion to predominate to a still greater extent.

2. Ammonia and bile salts possess the power of altering the physical state of aggregation of lecithin to such an extent as to permit of the conclusion that they can be of functional significance in altering the permeability of cell membranes. When we consider that this effect can be detected in the case of ammonia in a concentration of 1 : 500,000 and in the case of the bile salts in a con-

centration of 1 : 100,000, the importance of these substances in the regulatory activities of the body and the relation of the tissues to one another becomes apparent.

3. The ability of the tissue metabolites to combine with lecithin, as measured by the changes in the physical state of aggregation produced by their presence, is in some cases considerable, in other cases entirely lacking. Thus hypoxanthin, kreatin, kreatinin, adrenalin and ammonia salts show evidence of combination. Inosit is doubtful and urea is negative.

4. The amino acids show varying powers of combination. The dicarboxy acids, like acids in general, tend to increase the state of aggregation of lecithin.

5. The study of the action of drugs promises to yield results of interest and is to be continued.

In conclusion it gives us pleasure to thank Dr. P. A. Levene, Professor Otto Folin and Mr. F. C. Koch for kindly supplying us with some of the preparations used in this study.

5. THE FUNCTION OF THE BRAIN PHOSPHATIDS IN THE PHYSIOLOGICAL ACTION OF STRYCHNIN

W. KOCH AND H. T. MOSTROM

The physiological action of strychnin, as at present understood, may be briefly stated to be as follows: Strychnin is taken up by the nervous system, especially the cord, and acts on some mechanism lying between the sensory and motor apparatus in such a way as to decrease or remove inhibition. An explanation of the mechanism of the action of strychnin is destined therefore to give us a clearer notion of the phenomenon of inhibition. The method by which strychnin is taken into the nervous system has so far been only incidentally considered by Overton in the application, to other drugs, of his theory of the action of narcotics. As to the mechanism of strychnin action we have the work of H. v. Baeyer¹ demonstrating that it causes the discharge of stored oxygen in the nervous system. Until the oxygen has again been stored up in sufficient amount, no discharge can take place. Concerning the mechanism by which this oxygen is stored we have the suggestion of Fränkel² already expressed some time ago by one of us³ that the phosphatids by means of their unsaturated acids play a rôle in tissue oxydations, or in the attraction of the tissues for oxygen.

As all these observations point to the possible rôle of lecithin as an important factor in the action of strychnin, the following experiments were undertaken:

¹ Baeyer, H. v.: *Zeitschrift für Allgemeine Physiologie*, 1902, vol. 1, p. 265.

² Fränkel, S.: *Münch. medicin Woch.*, 1909, no. 47.

³ Koch, W.: *Zeitschrift für physiologische Chemie*, 1903, vol. xxxvii, p. 187.

TABLE I

Mechanism by which strychnin enters the nervous system

	WEIGHT OF FROG	TIME REQUIRED FOR TETANUS TO APPEAR
	<i>grams</i>	<i>in minutes</i>
1. <i>Control I.</i> 50 mg. strychnin, 1 drop conc. HCl made up to 50 cc. with water.....	22 21 23	3 2.5 3.5
2. <i>Control II.</i> 50 mg. strychnin, shaken for 1 hour with 50 cc. water.....	22 24 44 46	33.5 38 58 43
3. <i>Lecithin.</i> 50 mg. strychnin shaken for 1 hour with 50 cc. one per cent lecithin emulsion.....	25 28 49	4 5.5 3.0
4. <i>Kephalin.</i> 50 mg. strychnin shaken for 1 hour with 50 cc. one per cent kephalin emulsion.....	40 20	6 4
5. <i>Serum albumin (cryst.)</i> 50 mg. strychnin shaken for 1 hour with 50 cc. one per cent serum albumin	24 34	26 2 hours, no tetanus.

In each case the solutions after shaking were carefully filtered and 0.3 cc. injected into the dorsal lymph sac of the frog. The results are quite conclusive. The fact that the control does give tetanus after one-half hour is due to the very slight amount of the free alkaloid soluble in water alone.

The difference between serum albumin on the one hand and lecithin and kephalin on the other, is quite evident.

Mechanism by which strychnin combines with lecithin

Three factors may be concerned in bringing about the combination of strychnin with lecithin. (1) Traces of free acid present in the colloid as an impurity. (2) The acid hydrogen of the free hydroxyl of the phosphoric acid group in lecithin. (3) The dissociated fatty acid, especially oleic. The latter possibility is

suggested by the work of Kyes⁴ in the combination of cobra venom with lecithin. The following experiments were undertaken to determine these points:

TABLE II

	WEIGHT OF FROG	TIME REQUIRED FOR TETANUS TO APPEAR
	<i>grams</i>	<i>in minutes</i>
6. 50 mg. strychnin shaken for 1 hour with 50 cc. one per cent lecithin emulsion, which had been made alkaline with 3 cc. $\frac{N}{10}$ NH_4OH	40 49	2 7
7. By treating lecithin with iodine it is converted into an electro-positive colloid and can be precipitated by ammonia. The above experiment was repeated with this preparation which surely could contain no free acid.....	45 33	7 5
8. 25 mg. strychnin were shaken for 1 hour with 3 drops oleic acid in 25cc. water plus 3 cc. $\frac{N}{10}$ NH_4OH (the ammonia should be enough to neutralize any free acidity of the oleic acid) solution was distinctly alkaline to congo red.....	38 27	5 4.5
9. 25 mg. strychnin were shaken for 1 hour with stearic acid in 25 cc. water plus 3 cc. $\frac{N}{10}$ NH_4OH	38	35

The last experiment serves also as control. The strychnin combines with the lecithin therefore not on account of any free acidity, which was neutralized by the more strongly basic ammonia, but on account of some relationship to the oleic acid group. The cobralecithin of Kyes, however, which, according to Manwaring,⁵ is supposed to have had its oleic acid split off, still retains the power of taking up strychnin.

The above relationship to oleic acid suggests that strychnin not only enters the nervous system by combining with lecithin, but may also exert its physiological activity through the unsaturated groups of lecithin and cephalin. The following observation makes this more probable:

⁴ Kyes, P. Biochemische Zeitschrift, 1907, vol. iv, p. 99.

⁵ Manwaring W. H.: The Johns Hopkins Bull., 1910, xxi, no. 234.

When a drop of permanganate solution is added to an emulsion of lecithin, the color slowly disappears. If this lecithin emulsion has been previously shaken with strychnin, the color is discharged much more rapidly. Strychnin itself can be oxydized by permanganate, but not very readily under these conditions, so that a control with strychnin alone does not discharge the color of permanganate as soon as lecithin alone. The interpretation of this observation is, however, rather involved, and in order to demonstrate this action of strychnin more clearly the following experiment was devised:

In some studies on adrenalin the observation had been made that lecithin can act as a reducing agent, so that a solution of the free base adrenalin, which very quickly turns reddish and loses its activity on exposure to air, in the presence of lecithin will not show this change for several weeks, if the bacterial decomposition of the lecithin is avoided by sterilization. This is no doubt due to the fact that the lecithin by means of its unsaturated groups takes up the oxygen. If this is the function of lecithin in the tissues and the action of strychnin is to discharge this oxygen, it seems likely that in the presence of strychnin, lecithin or kephalin will not prevent the destruction of adrenalin. The following observations show this to be the case:

The following four solutions were made up:

- I. 50 cc. salt solution plus 5 mg. adrenalin (free base).
- II. 50 cc. salt solution plus 5 mg. adrenalin, emulsified with 500 mg. kephalin.
- III. 50 cc. salt solution plus 5 mg. adrenalin plus 50 mg. strychnin, emulsified with 500 mg. kephalin.
- IV. 50 cc. salt solution plus 5 mg. adrenalin plus 50 mg. strychnin.

The solutions were sterilized and allowed to stand with cotton stoppers. After ten days they were examined.

Blood pressure test. To determine the relative amounts of adrenalin left in these solutions the method of dilution was made use of.

Solution I and IV were negative when diluted six times.

Solution II was just negative when diluted forty-eight times.

Solution III was just negative when diluted twelve times.

Although in the presence of strychnin and kephalin (III) as much adrenalin had not been oxydized as in the controls (I and IV) the oxydation had proceeded much more rapidly than with the kephalin alone (II).

These observations were further confirmed by the color change of the solution. Solution II was the only one which had not turned reddish. The test on the enucleated frog's eye were also in harmony with the above observations. The experiment was repeated with lecithin instead of kephalin with practically the same result.

SUMMARY

We may conclude then from the observations here recorded, that: The central nervous sustem, especially the cord, by its high phosphatid content, is enabled to pick the strychnin out of the blood stream on account of the affinity of the lecithin and kephalin for the strychnin as compared to serum albumin. The strychnin probably enters into the combination with lecithin through some relation to its unsaturated fatty acid group (oelic acid).

Strychnin interferes in such a way with the normal relation of these unsaturated fatty acids groups to oxygen as to bring about a more rapid transfer to any easily oxydisable substance. (Under the conditions of these experiments, adrenalin.)

In conclusion, it gives us pleasure to thank Mr. F. Koch for a generous supply of adrenalin (free base) and Mr. H. C. Corper for a sample of crystallized serum albumin (human).

SOME OBSERVATIONS ON THE PHYSIOLOGICAL ACTION OF SODIUM CHLORIDE

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GENERAL REMARKS

Physiological salt solution. For the study of nerve and muscle of the frog after their separation from the living body it is desirable to preserve them in such a liquid medium as would prevent the drying of the tissues and at the same time exert no chemical action upon them. The observations of Koelliker¹ and others that nerve and muscle preserve their irritability and appearance for many hours in a solution of sodium chloride of a concentration ranging between 0.5 per cent and 1 per cent was therefore a timely discovery at the middle of the last century when investigations upon the behavior of nerve and muscle of the frog formed one of the chief studies in physiology. Henceforth sodium chloride in an 0.5 per cent solution was considered as an *indifferent* solution. Later, on the basis of the investigations of Nasse² who determined the optimum concentrations of various salts for the preservation of the irritability of tissues, sodium chloride was used in solutions of 0.6 per cent, this being considered as the optimum concentration for this salt. Hermann³ designated this solution as "physiological water." The term physiological salt solution became especially popular after the observations made in experimental physiology as well as in practical medicine that transfusions with this solu-

¹ Koelliker, Würzburger Verhandlungen, vii, 145, 1857, and ix, 15, 1859.

² Nasse, Pflügers Archiv, ii, 114, 1869.

³ Hermann, Handb. der Physiologie, Bd. i, Theil 1, s. 104.

tion are capable of saving the life of animals and man, endangered by profuse hemorrhage. At the end of the eighties when the problem of osmosis forged its way into biology our solution began to be designated as isotonic; the solution to be "indifferent" had to be isotonic with the serum of the blood. It was recognized at the same time that 0.6 per cent was only isotonic with the serum of the frog, and was therefore "indifferent" or "physiological" only for the tissues of this animal. By various methods it was established that for mammals the salt solution, to be isotonic with the serum of these animals, has to have a higher concentration. The consequences of these new conceptions found their way into practice only slowly, and even to this day the subject is frequently handled in a loose fashion. Physiologists employ generally concentrations lying between 0.9 per cent and 1 per cent equally for all kinds of higher animals. In the studies of immunity and in experimental pathology frequently a concentration of 0.85 per cent is being used for all kinds of mammals and birds. In medical practice we still meet quite often the "normal saline" of 0.6 per cent.

With the introduction of the osmotic factor our solution did not lose its "indifferent" character. On the contrary the original empirical observations seemed to have now received apparently a scientific basis: when a solution of sodium chloride is isotonic with the serum of an animal it is indifferent to its tissues because it then neither gives nor takes up fluid to or from these tissues. This implies, that sodium chloride acts on the tissues by the physical property of osmosis only; that chemically it is indifferent. However, in the course of various studies several facts gradually came to light which apparently did not harmonize with the idea of the indifference of sodium chloride. Kronecker and Stirling⁴ found that a frog's heart ceased beating in a pure physiological salt solution. Ringer⁵ discovered that frog muscles begin to twitch in such solutions and Locke⁶ found that it predisposes them to contractures and increases otherwise their irritability. Cars-

⁴ Kronecker and Stirling, *Ludwigs Festschrift*, 1874, 173.

⁵ Ringer, *Jour. of Physiology*, vii, 291.

⁶ Locke, *Pflügers Archiv*, Bd. 54, 501, 1893.

law⁷ and especially Locke⁸ discovered that a pure "physiological" salt solution abolishes the indirect muscle irritability by paralyzing the motor nerve endings. We thus see that a solution of sodium chloride which from the pure physical, osmotic point of view is indeed indifferent, is nevertheless capable of exerting abnormal influences in various directions. However, Ringer⁹ as well as Locke¹⁰ has discovered that all the above mentioned deviations from the normal occurring in the pure solution of sodium chloride can be corrected by the addition to the solution of small quantities of calcium. This fact permitted the interpretation that the deviations occur not on account of any special action of the sodium chloride, but merely on account of the absence of calcium. Indeed Howell,¹¹ who became interested especially in the exciting action of calcium upon the heart, went on record as late as 1899 with the statement that "sodium chloride seems to be essential only in preserving the osmotic relations between the tissues and the surrounding liquid." A few years later, however, Loeb¹² made the observations that the *Fundulus*, a marine fish, which is capable of living in distilled water perishes in a pure solution of sodium chloride; furthermore the addition of calcium to this solution deprives it of its poisonous influence. In this case the osmotic action of the sodium chloride solution could not come into consideration, since even pure water is harmless to the animal. The harmfulness of sodium chloride therefore must be due to some specific chemical action of the sodium chloride, which action, however, can be neutralized by the addition of calcium. Here was a definite instance in which sodium chloride proved itself to be chemically not indifferent to living tissue. Furthermore, since in this case the chemically injurious action could be corrected by the addition of calcium, the assumption is at least permissible that the other above mentioned injuries to the tissues caused by

⁷ Carslaw, *Archiv für Physiologie*, 1887, 429.

⁸ Locke, *Centralblatt für Physiologie*, viii, 166, 1894.

⁹ Ringer, *Jour. of Physiology*, iv, 29 and 222, and vii, 291.

¹⁰ Locke, *Centralblatt für Physiologie*, l. c.

¹¹ Howell, *American Jour. of Physiology*, ii, 47, 1899.

¹² Loeb, *American Jour. of Physiology*, iii, 333, 1900.

the immersion in pure solutions of sodium chloride are also due to the direct chemical action of the sodium chloride and that the further favorable effect produced by the addition of calcium does not come simply from the supply of a deficiency, but it means that an agent was applied which is capable of neutralizing the injurious action of sodium chloride. From these observations Loeb drew, with reference to the significance of the sodium ion two conclusions: First, that it exerts upon the tissues a definite ion action and, second, that this action is always in the direction of increasing the irritability. The latter conclusion became the subject of a good deal of discussion in which, however, we are not here concerned. The first conclusion, however, is now practically generally accepted. There is at present no one who is willing to stand sponsor for the claim that sodium chloride exerts only a physical, an osmotic, and not also a chemical action on living tissues. Sodium chloride solution, even in "physiological" concentrations has ceased to be an "indifferent" medium.

In the studies upon the comparative toxicity of the chlorides, the present writers¹³ arrived at the conclusion that the toxicity of magnesium, calcium, potassium and sodium for any living tissue stands in inverse proportion to the amounts in which these ions are present in the lymph surrounding these tissues. The ion which is represented in the lymph in the smallest amount is most poisonous and the one which is represented in the largest amount is the least poisonous. From this it would follow that an ion begins to exert an abnormal or toxic action on a living tissue as soon as it is present in the bathing medium in a quantity which exceeds that in which it is present in the normal blood and lymph of that tissue. The quantity of sodium chloride in the solution in which the frog muscle begins to twitch, or the frog's heart stops beating or the motor nerve endings lose their conductivity probably exceeds that which is normally present in the lymph of these tissues of the frog.

Salt action. In the above mentioned experiments upon the tissues of the frog, sodium chloride is capable of exerting a definite

¹³ Joseph and Meltzer, this JOURNAL, i, 1, 1909.

abnormal action even when the excess in quantity is comparatively very slight, because the tissues experimented upon are deprived of their natural medium and do not possess therefore sufficient calcium (and potassium) to neutralize the excess of sodium. It is different, however, when solutions of sodium chloride are introduced directly into the circulation. It is known that fairly large doses of sodium chloride can be injected intravenously into dogs without producing any definite untoward symptoms. In our¹⁴ experiments we have never seen any harmful effects following the intravenous injection of 30 cc. and even more of a molecular solution of sodium chloride per kilo animal. Assuming even that a part of the injected salt is rapidly eliminated again, there surely remains in the blood for some time an excess of it, large enough to be deleterious to the vital tissues through which it circulates. Probably it is the presence in the blood of calcium (and potassium) in quantities sufficient to neutralize the excess of sodium which inhibits the production of pathological manifestations.

There is however a limit to the innocuousness of sodium chloride even by the transfusion method. When the injection of the salt solution is continued, a period is soon reached when tremors and twitchings set in, which gradually develop into convulsions. These terminate in paralysis which leads up gradually to the death of the animal. Such manifestations, however, occur only when the injected sodium chloride solutions are strongly hypertonic. These effects of hypertonic solutions of sodium chloride were designated by pharmacologists as *salt action*, meaning thereby that the phenomena were produced by the withdrawal of water from the tissues by the salt. We meet here again therefore with the contention that the abnormal reaction of the tissues to the injection of sodium chloride is due to the physical effect of osmosis. While for the isotonic solutions of sodium chloride it is now, as stated above, quite safely established that it exerts also a definite chemical effect upon the living tissues, for the effects of the hypertonic solutions of the same salt the claim of some pharmacologists that it is purely a physical action has hardly been seriously questioned.

¹⁴ Joseph and Meltzer, l. c.

In the above mentioned investigation upon the comparative toxicity of various ions we¹⁵ studied the effects of intravenous injection of sodium chloride in molecular solution upon twelve dogs. We then observed among other things, that "when the infusion approached the end of the fourth hundred (cc. of the molecular solution), for dogs of 7 or 8 kgms., muscular twitchings began which gradually developed into strong clonic convulsions." The convulsions never terminated directly in the death of the animal. Without any exception there was an interval between the convulsions and the death of the animal, during which interval respiration and pulse were sometimes still favorable and reflexes active. Gradually all began to fail, the respiration as a rule stopping at least two or three minutes before the heart. In the discussion of these results we touched upon the question, how far these phenomena may be due also to some chemical action of the sodium chloride, and offered then the following two suggestions: 1, that the twitchings might be similar to the twitchings of frog muscles occurring even in isotonic solutions of the salt, and, 2, that the cessation of the respiration before the heart beat might be due to a curare-like action of the sodium chloride upon the respiratory motor nerves, which would again be similar to the effect of isotonic solution of sodium chloride upon the endings of the motor nerves of the frog (Locke¹⁶).

EXPERIMENTAL OBSERVATIONS

In the following we wish to report briefly the results of a series of experiments which we carried out recently for the testing of the two above mentioned hypotheses. The experiments were again made on dogs, twenty-six in number. In most of the experiments the sodium chloride was employed in molecular solutions. All the injections of this solution were given through the left external jugular vein, the solutions running from a burette under constant pressure. In many cases the animals were tracheoto-

¹⁵ Joseph and Meltzer, l. c.

¹⁶Locke, *Centralbl. für Physiologie*, l. c.

mized, to facilitate the respiration. All operations were performed under ether anesthesia which was discontinued before starting the infusions.

The first hypothesis was that the twitchings developing in mammals after an infusion of a considerable quantity of a strongly hypertonic solution of sodium chloride are similar to the twitching of frog muscles when immersed in a solution of the same salt. The twitchings of the frog muscle set in the more promptly and are the stronger the higher the concentration of the solution. We could assume that in the course of an infusion into dogs when the concentration of the sodium within the blood reaches its first degree of effectiveness the muscles of the animal begin to twitch; with continuation of the infusion and the increase of the concentration the twitches grow into convulsions. In frog muscles sodium chloride produces twitchings even when the muscles are taken from curarized animals. The twitchings therefore must be of myogenic origin. If we assume that the mammalian twitchings are identical with that of the frog muscles, we would have to assume that the twitchings and convulsions produced in dogs by the sodium chloride have their origin also in the muscle tissue and not in the nervous system. This point offered a simple means of testing our hypothesis; we had only to establish whether the interference with the nervous system interferes with the appearance of the twitching and the convulsions. There was, however, yet another simple method for testing the question of the identity of both kinds of twitchings. We know now that the addition of a minute quantity of calcium to the solution of sodium chloride is sufficient to stop promptly the twitchings of frog muscles. We had then to test whether the infusion of calcium will also interfere with the appearance of the twitchings in dogs. We employed both methods and shall speak of the last named, first.

For these experiments we have used calcium chloride in $\frac{M}{8}$ solution which was permitted to run into the right jugular vein while the sodium chloride continued to run into the left. The infusion of calcium was started after the tremors and fibrillary contractions had set in and were well defined. We shall say here a few words about the development and course of these contractions.

They are very slight at the beginning and appear only in a part of the body. Gradually they spread over the entire body and become stronger. Soon, some of the tremors grow into strong convulsions which make the table shake. During a strong convulsive movement no tremors can be noticed but they are manifested at the intervals between the convulsive attacks. With the increase in frequency of the convulsions the fibrillary contractions seem to decrease. After reaching a certain height in strength and numbers the convulsions begin to decrease again, first in their number and soon also in strength. The decrease develops fairly rapidly.

We may say now that in every experiment in which the infusion of calcium was tried, the twitchings and convulsions brought on by sodium chloride ran their usual course without being affected by the calcium. Here we have a definite differentiating point between the two forms of twitchings: whereas the twitchings of the frog muscle brought on by sodium chloride subside promptly upon the addition of a very small amount of calcium chloride, the twitchings and convulsions of the dogs brought on by the infusion of sodium chloride are not apparently affected in the slightest by a simultaneous infusion of 60 cc. and more of an $\frac{M}{8}$ solution of calcium chloride.

The other method of testing also gave unmistakable results. It was tested in two ways. In the first place, in many experiments one sciatic nerve was cut for a purpose which will be mentioned later. In these experiments, it was evident that the leg, the sciatic nerve of which was cut, never took part in the tremors or in the convulsions. If the twitchings had been myogenic in origin, as is the case for the twitchings of frog muscles, cutting of the sciatic nerve could not have interfered with their appearance. The twitchings of the infused dogs depend apparently on the nervous system. This was demonstrated in a still more striking way in an experiment in which the posterior part of the spinal cord was destroyed. The contrast between the strong convulsions of the anterior part and the paralytic quietude of the posterior part of the animal was striking and instructive. We shall reproduce here a greatly abbreviated protocol of this experiment which will illustrate at the same time some other statements made in this paper.

Experiment 16, September, 26, 1910

Young male dog, 7050 gms., in good condition. 10:00 a.m.: Etherized, tracheotomized, cord exposed and cut at about the 7th dorsal vertebra. Posterior part of cord completely destroyed by punching it out with a brass tube sharpened at the end. Animal allowed to recover.

1:25 p.m. Etherized again and cannulas inserted into jugular vein and carotid artery. Blood-pressure varies between 100 and 130 mm. mercury; respiration good; shivering of whole chest (no fibrillary tremor), hind part quiet. Ether discontinued and started infusion of NaCl in mol. solution.

2:40 p.m. 95 cc. NaCl in. Blood-pressure 142 mm., no changes.

3:18 p.m. 240 cc. in. Definite twitching of forelegs and shoulders, hind legs perfectly quiet.

3:45 p.m. 395 cc. in. Powerful convulsions, involving all anterior parts of body; hind parts perfectly quiet.

3:55 p.m. 470 cc. in. Convulsions still very strong, blood-pressure between 180 and 190 mm.

4:12 p.m. 560 cc. in. Blood-pressure between 160 and 196, convulsions moderately strong and infrequent.

4:17 p.m. Convulsions nearly all gone, blood-pressure between 108 and 170, heart irregular, respiration not very satisfactory.

4:21 p.m. Convulsions all gone, respiration shallow, heart more regular now. Blood-pressure varies between 80 and 140.

4:24 p.m. 600 cc. NaCl in; stop. Respir. still shallow, heart more regular now, blood-pressure 60-120 mm.

4:26 p.m. Respiration gone, heart beats well, blood-pressure, 40-58.

4:28 p.m. Blood-pressure 25 mm. Hg, only an occasional heart beat.

Autopsy. No fluid in thorax, about 40 cc. in abdominal cavity, no pulmonary oedema. Marked oedema in tissues around the kidneys, cortex of kidney very pale. Fluid gushed from kidney when cut open.

Total quantity of urine passed during infusion 400 cc., contained no sugar at any time.

The experiments then have shown that the twitching and convulsions appearing in dogs after intravenous infusion of strongly hypertonic solutions of sodium chloride are not affected by a simultaneous injection of calcium chloride, and that the muscles do not twitch if the corresponding section of the cord is destroyed or the motor nerve cut. This means in the first place that the

hypothesis is not sustained; the twitchings produced in mammals have nothing in common with the twitchings of the frog muscle. The experiments brought out at the same time one positive result regarding the situation of the cause of the twitchings and convulsions in mammals; they undoubtedly originate in the spinal cord.

The second hypothesis was that the paralysis of the respiration might be due to a curare-like action of the sodium chloride upon the motor nerves of the respiratory muscles and would be identical with the action of sodium upon the endings of the motor nerves in frogs. It could be assumed that sodium chloride, like curare, affects the motor nerves of the respiratory muscles much later than that of the other skeletal muscles. Hence the earlier subsidence of all convulsions and the subsequent paralysis of respiration.

We shall recall here the above mentioned fact that the paralyzing action of sodium chloride upon the motor nerve endings in frogs is promptly reversed by the addition of a small quantity of calcium chloride. In our present experiments we have tested the action of an intravenous injection of calcium chloride upon the respiratory paralysis and found that it had not the slightest effect.

Furthermore the hypothesis in question was tested in a direct way. In the first place the peripheral end of the sciatic nerve was cut and stimulated during the paralytic stage. It was found that even then the corresponding muscles responded promptly. In many instances the sciatic nerve was cut at the beginning of an experiment and the peripheral end was placed in a Sherrington¹⁷ electrode over which the wound was closed. The nerve was then tested at various times during the experiment. It was found that in many cases the nerve lost very little from its original irritability. In some instances the loss was perceptible; but apparently not greater than that which may occur in some cases under normal conditions in the course of a few hours after the section of the nerves. We should add that nerves which in our experiments lost perceptibly from their original irritability did not recover anything of the loss by an infusion of calcium chloride.

Our second hypothesis also did not stand the experimental test. From the above we must conclude that the cessation of the

¹⁷ Sherrington, *Journal of Physiology*, xxxviii, 382, 1909.

convulsions at the end of a prolonged infusion of a hypertonic solution of sodium chloride into mammals and the subsequent paralysis of the respiration are not caused by a paralysis of the motor nerve endings.

It is needless to say that while these experiments have disposed of the two hypotheses which we offered in behalf of the assumption that the phenomena of motor excitation and paralysis following a prolonged infusion of hypertonic solutions of sodium chloride are caused, partly at least, by definite chemical (or ion) actions, they have, of course, not disposed of the assumption itself, into a further discussion of which we shall, however, not enter here.

In view of the surprising scarcity of original work on the effects of intravenous injections of hypertonic solutions of sodium chloride it will not be amiss to bring to record very briefly and aphoristically some observations we have gathered in our present series of 26 dogs.

It is possible that the infusion of the solution produces at the beginning some excitement of the animal with a marked increase of respiratory activity. At least this was the case in many of our experiments. However, since in many of the experiments the starting of the infusion was coincident with the awakening of the animal from the ether anesthesia we could not be sure what share this state might have had in the excitement. At any rate the excitement passed off very soon. On the contrary most of the animals soon became very quiet. During the inflow of the first 20 to 30 cc. (per kilo) of the solution the condition of some of the animals was marked by nothing so much as by unusual quietness. At this period many animals were frequently snoring, although the lid reflex was very active and the animal seemed to be otherwise wide awake.

Regarding the tremor and convulsions, we described above their development and course. We shall add here that the convulsions, even if they are very strong, differ distinctly from those seen in strychnine poisoning, tetanus, epilepsy, and eclampsia. In the first place they *rarely come to a steady tonic contraction, a tetanic attack*. In the second place the *clonic convulsions do not show an orderly alternation of contractions between antagonistic muscle*

groups. As a rule no group of functionally connected muscles seems to contract as a unit. The convulsive movements are perhaps described best as *choreiform*.

We have made a few experiments in which NaCl was infused in bimolecular (2M) and tetramolecular (4M) solution. In each case the course was marked by the same stages observed to occur when the salt was administered in molecular solutions; but the stages were of shorter duration. In the experiments with bimolecular solutions the animals died with about half of the quantity, and in those with tetramolecular the animals died with about one fourth of the quantity which caused death by molecular solution. This would seem to mean that in all acute cases death was brought about by the same amount of salt.

In some experiments we have studied the effect of the infusion upon blood-pressure. It goes without saying that there was a marked rise of pressure during the convulsive stage. However the *pressure began to rise even before any fibrillary contractions were noticeable*.

The convulsions have been controlled to a marked degree by intravenous injections of doses of magnesium chloride which were insufficient to impair the respiration. Also small doses of potassium cyanide were capable of quieting considerably the convulsive movements.

It has been stated by some writers that toxic doses of sodium chloride cause the development of pulmonary oedema. In our experience this was true only for a small minority of the experiments. In the majority of the cases *there was no oedema at all or the oedema was very slight*.

In the present series of experiments with molecular solutions the urine collected rarely exceeded the quantity of the infused solution. In the experiments with higher concentrations the quantity of urine always exceeded that of the solution injected.

In most of the experiments the urine contained no sugar. And even in the cases in which sugar was present at the beginning it disappeared later, at least in most of these cases.

The following table will illustrate some of the foregoing statements:

TABLE

Showing the concentration and quantity of sodium chloride injected, the quantity of urine collected with the presence or absence of sugar, and the findings at autopsy with regard to pulmonary edema.*

NUMBER OF EXPERIMENT.	BODY WEIGHT.	CONCENTRATION OF SALT USED.	TOTAL NUMBER OF CC. NaCl INJECTED TO DEATH.	TOTAL CC. URINE COLLECTED.	SUGAR TEST. (Fehling's)	PULMONARY EDEMA AT AUTOPSY.	REMARKS.
1	5250	$\frac{M}{I}$	500		negative	tremendous edema.	
3	4200	$\frac{M}{I}$	320		not tested		
4	4000	$\frac{M}{I}$	250		strong reduction	considerable edema	
5	4200	$\frac{M}{I}$	405	380	strong reduction	marked edema	
6	6000	$\frac{M}{I}$	550	500	moderate reduction	slight edema	
7	5650	$\frac{M}{I}$	360		negative	slight edema	
8	6800	$\frac{M}{I}$	740	875	negative	slight edema	
9	5800	$\frac{M}{I}$	455	300	negative	no edema	
10	5500	$\frac{M}{I}$	475	450	negative	very slight edema	
11	5250	$\frac{M}{I}$	430	440	reduction	no edema	
12	6750	$\frac{M}{I}$	630	250	negative	slight edema	
13	5900	$\frac{M}{I}$	450	625	negative	no edema	cord cut at 1st dorsal
14	6500	$\frac{M}{I}$	580	260	negative	slight edema	cord cut at 2nd cervical
15	6050	$\frac{M}{I}$	410	220	negative	no edema	cord cut at 2nd cervical
16	7050	$\frac{M}{I}$	600	220	negative	no edema	cord destroyed below 7th dorsal
17	6690	$\frac{M}{I}$	370		negative	no edema	
18	5650	$\frac{M}{I}$	450	475	negative	no edema	
22	4650	$\frac{4M}{I}$	85		no test	marked edema	
23	4450	$\frac{4M}{I}$	83	185	negative	slight edema	
24	4800	$\frac{4M}{I}$	102	190	faint reduction	slight edema	
25	6800	$\frac{2M}{I}$	330	425	negative	no edema	
26	6650	$\frac{2M}{I}$	220	575	slight reduction	no edema	
27	7700	$\frac{2M}{I}$	317	510	negative	no edema	

*Three experiments are omitted, because the sodium injection was stopped some time before death of the animal.

THE DISTRIBUTION OF HAEMOLYSINS AGGLUTININS AND POISONS IN FUNGI, ESPECIALLY THE AMANITAS, THE ENTOLOMAS, THE LACTARIUS AND THE INOCYBES

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INTRODUCTION

It has previously been suggested that the methods which have been developed for the study of *Amanita phalloides*, *Amanita muscaria*, *Amanita rubescens*¹ and the species belonging to the genus *amanita*, in the application of which it was found that the poisonous or non-poisonous qualities of these fungi could be determined by laboratory tests, might be applied to other genera in the hope of establishing their properties more accurately than by the older expedient of eating small quantities of the unknown plants and watching for the appearance of unpleasant or disastrous consequences. The opportunity of testing this hypothesis, by the examination of a large number of agarics, including representatives of nearly all of the genera growing in the United States, has been afforded me by the kindness of several members of the Boston Mycological Club, who have sent me, from time to time, carefully preserved and accurately identified specimens. I am particularly indebted to Mrs. E. B. Blackford, Mr. Simon Davis, Mr. L. C. C. Krieger and Mr. George E. Morris who have provided me with material exhibited at the Club meetings and with material from their private collections. Only by the coöperation of these well trained mycologists was it possible to continue the investigations previously started on the *amanitas* and extend them to other groups of agarics. Especial emphasis has

been laid upon the careful study of species known to be poisonous to man, and of species which were reputed to possess deleterious properties or which from their close resemblance to well-known poisonous forms were open to suspicion. In addition, a number of plants which were known to be edible were investigated to determine whether they also harbored principles poisonous to animals on subcutaneous inoculation. Finally a careful study of the literature has been undertaken and especially the testimony of trained mycologists has been gathered to try and determine with some accuracy which of these fungi are known to be poisonous and how many of them have actually been eaten without harmful result. In this latter task I have taken advantage of the very valuable records of the Boston Mycological Club and I am greatly indebted to the members of this organization for information concerning the properties of many of the species described in this paper. The great diversity of opinion among mycologists as to the proper identification of our rarer mushrooms, and the difficulties presented by a somewhat confusing nomenclature of fungi in general has made it necessary that investigations of this character should be carried out with a constant appeal for information to some responsible organization, if the results obtained are to be of permanent value in establishing the properties of American fungi.

METHODS

The material submitted for examination was all studied by the methods already described. The thoroughly dried plants were macerated in water or physiological salt solution in about the proportion of 1 gram to 10 cc., filtered after 18 hours preservation on ice, neutralized, when acid, with sodium bicarbonate, and the raw extract thus obtained tested upon red blood corpuscles to detect the presence of haemolysins or agglutinins. The blood of rabbits, guinea pigs, fowls, and man was employed indiscriminately for this purpose as it has been found that nearly all varieties of corpuscles are sensitive to the action of the haemolytic and agglutinating substances in fungi. The extracts were then

heated to a temperature of 60–65° C. for half an hour and again tested upon blood to determine whether the haemolysins and agglutinins which might have been previously found were destroyed at this temperature, that is, whether they were thermo-labile or thermo-stabile. The heated material was then injected subcutaneously into guinea pigs, usually in quantities varying from 3 to 5 cc., according to the body weight. Whenever these heated extracts proved to be poisonous to guinea pigs, rabbits were also inoculated with proportionate quantities, and in all those species which are reputed to be poisonous and in species closely related botanically to well known poisonous forms, both guinea pigs and rabbits were inoculated originally with the heated extracts, provided the amount of material sufficed for this purpose.

As far as possible when the fungi thus examined were found to contain definite poisonous principles, the extracts were boiled half an hour and injected into guinea pigs to determine whether the poisonous substances are destroyed by boiling or not and finally some of the fungi which were at my disposal in abundance, such as *Amanita muscaria*, *Amanita phalloides* and *Lactarius torminosus*, which are known to be fatal or intensely poisonous to man on ingestion, were *cooked* and the juice thus obtained tested upon corpuscles and upon animals. In these latter tests, the conditions under which mushrooms are prepared in the kitchen for the table were imitated as far as possible. The plants were either cooked whole or cut up into fairly small pieces, enough water being added to prevent them from burning. This material was heated in a tin cup over a free flame, and for half an hour was allowed to gently simmer, boil freely or violently. The temperature maintained in cooking the fungi in this way was naturally found to vary greatly depending upon the strength of the flame, but in general it ranged from 75° to about 95°. After cooking for half an hour, the "mess" was thoroughly ground in a mortar, put on ice over night and then an extract made and tested upon blood and for toxicity.

By these methods of examination it is possible to obtain a great deal of information about the properties of fungi. The presence or absence of haemolysins and agglutinins may be determined

with some accuracy and the action of the heated and boiled extract upon animals may be established. The most difficult part of the problem lies in the determination of the toxicity of the extracts. Many of the rabbits provided the laboratory are infected with coccidia, and many of the guinea pigs die of spontaneous infections if they are kept long in confinement. Whenever possible, therefore, extracts which were found to be poisonous were again administered to animals for confirmation of the previous findings. While the majority of species which are clearly poisonous exhibit their action in a few days, producing an acute intoxication, a certain number cause a chronic emaciation, from which the animals die in 18 to 20 days. Both rabbits and guinea pigs were therefore kept under observation for a period of four weeks, beyond which time fungus intoxications have not been found to develop. Finally the observations made upon the various species examined by these methods have been compared with the information available as to the consequences which follow their ingestion in man, and the attempt made to establish the properties of the various species.

THE AMANITAS—WHITE-SPORED

AMANITA PHALLOIDES *Bulliard*

It has now been shown that the "white" or "deadly" amanita contains a powerful haemolysin which acts upon a great variety of corpuscles, is destroyed by heating to 60–65° C. for half an hour, and has, according to Abel and Ford (2), the chemical composition of a glucoside containing C = 48.93, H = 6.08, N = 10.83, S = 1.94, O = 32.22. This substance from its destruction by heat and its susceptibility to the action of artificial gastric juice can not be held responsible for the symptoms seen in poisoning with the fungus in man. The species apparently owes its toxicity to the *Amanita-toxin* a method for the isolation of which has been worked out by Schlesinger and Ford (3), although its presence in alcoholic extracts of the plant was clearly recognized by Kobert (4).

Identical with *Amanita phalloides* in all particulars are *Amanita virosa* and *Amanita verna* while a number of other amanitas such as *Amanita porphyria* Albertini and Schweinitz, *Amanita strobiliformis* Vittadini,

Amanita radicata Peck, and *Amanita chlorinosma* Peck, while devoid of haemolysins, produce an intoxication in animals like that due to the heated *Amanita phalloides*, and apparently owe their toxicity to the presence in small amounts of the *Amanita-toxin* (5).

It has previously been shown by Ford (6) that solutions of the *Amanita-toxin* suffer some loss in toxicity on boiling, but Schlesinger and Ford subsequently pointed out that this diminution in toxicity is not great. Experiments were undertaken to determine whether *Amanita phalloides* cooked in the manner already mentioned for half an hour will retain its poisonous action. Ten grams of typical plants collected during the summer of 1910 at Saunderstown, R. I., were cooked half an hour in 100 cc. of water. During this time the temperature varied from 70–98° C. The fungi were then macerated in a mortar, placed on ice over night, the juice expressed and filtered, nearly 40 cc. of a dark brown fluid with the characteristic odor of this plant being obtained. This extract was quite devoid of haemolytic activity, but was highly toxic to animals. Five cubic centimeters killed a guinea pig weighing 385 grams over night, the same amount killed another guinea pig weighing 420 grams in four hours, and a rabbit weighing 1035 grams succumbed within 18 hours to a dose of this character. It is evident therefore that *Amanita phalloides* while losing its haemolytic activity in ordinary cooking, retains its toxicity practically unimpaired, a fact which is in close agreement with the clinical observations that death from *Amanita phalloides* intoxication can in the majority of instances be traced to the use of the cooked fungi.

AMANITA MUSCARIA *Linnaeus*

This poisonous mushroom, known popularly as the “yellow” or the “fly” amanita, owes its toxicity to the crystalline substance muscarin, first obtained by Schmiedeberg and Koppe (7), formerly classed with the alkaloids but now grouped with the ammonia derivatives. Its composition is usually given as $C_5H_{15}NO_3$. The characteristic action of *Amanita muscaria* and of the muscarin extracted from this plant, is upon the nerve centers, the animals dying in convulsions. Ingestion of the fungus in man is also followed by convulsions from which recovery may take place, especially when the free use of atropine is employed.

In addition to muscarin, a second poison in this plant has been hypothesized by Harmsen (8) to explain the toxicity of the extracts when the muscarin is completely neutralized by atropine and I have recently

shown that this species contains also an haemolysin and an agglutinin (9). Beyond the fact that the haemolysin is soluble in alcohol little was learned of its properties, but the agglutinin was shown to belong to the glucosides. At least proteid could be removed from it by uranyl acetate in alkaline solution, and the addition of uranyl acetate in acid solution, of ammoniacal cupric acetate, and of basic lead acetate gave precipitates which when decomposed exhibited the characteristic agglutinating action upon corpuscles and gave the chemical reactions for a sugar-containing substance.

Subsequent examination of a number of typical *Amanita muscaria* has revealed the constant presence of this agglutinin and the introduction of small quantities of the extracts subcutaneously always produced the death of animals in typical convulsions. In some plants indeed, which from meteorological conditions differed markedly from the type form of *Amanita muscaria*, the presence of this agglutinin and the characteristic reaction upon animals gave the clue to the identification of the specimens. One may therefore very properly raise the question whether the presence of muscarin and possibly this agglutinin also may not be regarded as essential characters of the species necessary for its proper determination.

Much confusion appears in the literature as to the edible properties of the "yellow amanita." It is regarded as deadly poisonous by mycologists and pharmacologists in general, but occasionally the statement is made that *Amanita muscaria*¹ may be eaten without fatal effects, and its use both as a condiment and as the source of an intoxicating beverage by the peasants of the Caucasus has never been clearly understood. While it may be possible that the *Amanita muscaria* grown in Russia is free from muscarin, the symptoms described from its use indicate that muscarin or some substance similar in action upon the nerve centers must be present. In order to determine the effect of heat upon this fungus about 7 grams of very light and dry specimens collected by Mr. Simon Davis and myself near Stow, Mass., were soaked in about 200 cc. of water and cooked over a Bunsen burner half an hour. The material was allowed to boil violently, the temperature ranging from 95° to 99° C. At the end of this time the fungi were further macerated, preserved on ice over night, an extract made and filtered. This extract

¹ It is stated also that the variety "*formosa*" of *Amanita muscaria* may be eaten with impunity. Further work is necessary to determine whether this variety contains muscarin.

had no action upon animals or upon blood corpuscles, both the agglutinin and the muscarin being broken down. Specimens from the same lot were now prepared in the usual way and their extract found to contain both substances, 5 cc. of an extract of 5 grams of material in 80 cc. of water killing a 270-gram guinea pig in two hours. This extract was then boiled in a water bath 15 minutes and half an hour, and in both instances proved fatal to guinea pigs in 5 cc. doses. We can only conclude therefore that under certain circumstances cooking *Amanita muscaria* may rob it of its toxicity, but can express no opinion as to the reason for this change. This experiment, however, may explain some of the divergent statements in the literature and the claims often made by mycologists that the plant may be eaten without disastrous consequences.²

AMANITA RUBESCENS *Persoon*

I have previously shown that *Amanita rubescens* or the "red amanita" is free from *Amanita-toxin* (10) although containing a powerful haemolysin which was probably to be classed with the glucosides. A number of other specimens of this species have been examined and found to contain this blood-laking substance. That it has no effect when ingested by man, is shown by the fact that *Amanita rubescens* in this country is definitely edible. It is so regarded by Atkinson (11), by McIlvaine and Macadam (12), and by the Boston Mycological Club and I was able to find several competent mycologists who agreed as to its properties and stated that they themselves had eaten it frequently.

The European *Amanita rubescens* is sometimes called edible and sometimes poisonous. Kobert (13) regards the Perlpilz (*Amanita rubescens*) as poisonous on the evidence of Dragendorf, but states that Leuba considers it non-poisonous. According to Kunkel (14), Krombholz calls it poisonous, Rabenhorst very poisonous and Huseman, edible. The question of proper identification of the species probably enters into the discussion as to the properties of the European plant of

² In some of the guinea pigs dead of *Amanita muscaria* intoxication, extensive hemorrhages in the wall of the stomach with considerable amounts of free blood in the stomach contents, were observed. This lesion is rarely seen in experimental intoxication with this fungus and hemorrhages are seldom described in fatal cases of "muscaria" poisoning in man. Apparently the plant contains some heat-resistant principle acting upon the vessel walls in addition to muscarin or the natural muscarin has some destructive power for the endothelium of the blood vessels, as well as its influence upon the nerve centers.

this name, but the testimony of mycologists in this country and the laboratory investigations which we have carried out with accurately identified specimens would seem to be conclusive and to indicate clearly that the American *Amanita rubescens* is to be classed with the edible mushrooms.

AMANITA SPRETA Peck

Previous examination of this amanita showed that it contained a small quantity of haemolysin, and a toxic substance probably identical with the *Amanita-toxin*. The fungus was classed provisionally with *Amanita phalloides*, *Amanita verna* and *Amanita virosa*. We have recently been able to examine a number of fairly fresh specimens of this plant. In all cases the extracts were actively haemolytic, quite as much so as the typical *Amanita phalloides*. In most instances the heated extracts were poisonous to guinea pigs producing both an acute and chronic intoxication, but no toxicity was observed for rabbits. Despite this fact, I should regard *Amanita spreta* as a poisonous plant to be grouped with *Amanita phalloides*, especially since the extract boiled half an hour produced an acute intoxication in a guinea pig, the animal dying in less than 18 hours.

Little is known as to the edible or poisonous qualities of *Amanita spreta*. It is regarded as poisonous by the Boston Mycological Club and by Atkinson, who states that it is "said to be poisonous."

AMANITA FROSTIANA Peck

Because of the resemblance of this species to *Amanita muscaria* it is regarded as poisonous by its discoverer, Peck, and by Atkinson (15). I have already shown by the examination of several specimens, that the species contains a thermo-labile haemolysin, but no muscarin, its extracts being without action upon animals. Recent analysis of other fungi of the same species confirms these earlier conclusions, the "frostiana" being free from muscarin and from other poisonous substances. As far as can be learned, its edible properties have not been tested.

AMANITA MORRISSII Peck

Several specimens of this species, which was discovered by Mr. George E. Morris and named by Peck (16), were provided me by Mr. Morris. The species contains a small amount of haemolysin, destroyed

at 60-65° C., and is poisonous to both guinea pigs and rabbits. When first examined it exhibited this action constantly, the animals dying of a chronic intoxication like that due to small doses of *Amanita phalloides*. Subsequently, eight or nine months after the first examination, the poisons had deteriorated somewhat, but a definite toxicity could still be demonstrated for both species of animals. It should be grouped, at least for the present with the "deadly amanitas." As far as can be learned its edibility has never been tested.

AMANITA CITRINA Persoon

Specimens of this fungus sent me by Mr. Davis and by Mr. Morris agreed in their main characters. All were free from haemolysin and agglutinin. When some of the specimens were first examined, they were found to be poisonous to guinea pigs and rabbits, producing both an acute and a chronic intoxication. Several months later the toxicity for guinea pigs was found unimpaired, but the extracts had lost their action upon rabbits. The boiled material was without action upon animals.

This fungus is called by Kobert (17) a yellow variety of *Amanita phalloides* and is said by him to be poisonous. He distinguishes also a yellow form of *Amanita muscaria*, a different fungus from the *Amanita citrina* of Persoon. Kunkel (18), however, found that fungi gathered by him for four years near Würzburg and identified as *Amanita bulbosa*, variety *citrina*, had no haemolysin but a weak definite toxic action upon mice and cats, during one season only. Abel and Ford (20) have recently reported the presence of an haemolysin in fresh specimens of fungi considered by them to represent the yellow form of the "phalloides." As with other species of amanitas, the nomenclature of this plant is very confusing and its identification is by no means always simple. It is possible that the plant regarded as *Amanita citrina* in one region would not be so identified in another locality. The different specimens I have examined correspond to the *Amanita citrina* of Persoon and their properties are identical. For the present, we must regard the plant as belonging to the group of amanitas containing the *Amanita-toxin*, but no haemolysin, and including such species as *Amanita porphyria*, *Amanita strobiliformis*, *Amanita radicata* and *Amanita chlorinosma*. It is regarded as a poisonous species by the Boston Mycological Club.

AMANITA CRENULATA Peck

Several specimens of this fungus were sent me and examined at various times. They agreed in their properties. The species is free from haemolysins and agglutinins. It is toxic to both guinea pigs and to rabbits, producing a chronic intoxication in both animals. Thus 3 cc. of an extract made from 3 grams in 30 cc. of water killed a 370-gram guinea pig in 15 days and 4 cc. of a similar extract killed a rabbit weighing 1250 grams in 27 days. Subsequently over a year after the specimens were gathered another extract in the same proportion was made and 3 cc. of this extract killed a 465-gram guinea pig in 13 days and 5 cc. produced an acute illness in a 2000-gram rabbit from which the animal apparently recovered, but developed a chronic intoxication, dying in 23 days. Finally the boiled extract in a 4 cc. dose killed a 270-gram guinea pig in 10 days.

The species evidently contains in small quantities a poison similar in its action to the *Amanita-toxin*. *Amanita crenulata* is said by McIlvaine and Macadam (21) to be edible. I have never been able to obtain any positive information that it has been eaten and should be inclined to group it with the amanitas containing no haemolysins or agglutinins, but small quantities of the *Amanita-toxin*.

AMANITOPSIS VOLVATA (Peck) Saccardo

This species, originally described by Peck (22) as an amanita, contains neither haemolysin nor agglutinin. Three cubic centimeters of an extract in the usual proportions killed a 300-gram guinea pig in seven days and 4 cc. killed a 1250-gram rabbit in 22 days. Several months afterwards, 3 cc. of a similar extract killed a 480-gram guinea pig in 11 days but the toxicity of the extract for rabbits seemed to have disappeared. Four cubic centimeters of the boiled extract, however, killed a 375-gram guinea pig in 14 days. In all cases the intoxication resembled that produced by other amanitas. This species is said to be edible by the Boston Mycological Club, on the basis of the statement of McIlvaine and Macadam (23) that the plant has a tender, delicate but not pronounced flavor. I have found but one instance of its use as food and then in very small quantities. It should probably be classed with the poisonous amanitas we have already considered and should not be eaten.

THE CLITOCYBES—WHITE-SPORED

Several species of clitocybe have been described, a number of them being reported in this country by Peck (24) and by Morgan (25). While the genus is usually regarded as edible, *Clitocybe candida* Bresadola and *Clitocybe laccata* Scopoli being possibly the best known in this respect, *Clitocybe illudens* or *Agaricus illudens* is a poisonous fungus. I have examined three different species.

CLITOCYBE ILLUDENS *Schweinitz*

The *Agaricus* or *Clitocybe illudens* has long been known to cause transient illness in man, and Farlow (26) has recently again called attention to its poisonous qualities. The symptoms following its ingestion consist of violent gastro-intestinal disturbances with vomiting and diarrhoea, accompanied by great prostration. The illness lasts but a few hours and at the end of a day or two the patients are restored to normal health. No deaths are recorded from its use as food even in considerable quantities. The species grows in large clumps, about the bases of old stumps, has a rich saffron color and is strongly phosphorescent, being sometimes called "Jack my lantern."

The specimens examined were free from substances acting upon the blood cells, but contained a poison producing an acute intoxication in guinea pigs. Following inoculation of small quantities such as 3 cc. of an extract made in the usual proportions the animals died in from one to seven days, although sometimes a chronic intoxication developed the animals dying after an interval of 15 to 16 days. At autopsy there were no particular lesions. Rabbits apparently were not affected by this poison. The dried material retained its toxicity for over a year, but boiling half an hour destroyed it. This species is regarded as harmful, but not deadly by the majority of mycologists.

CLITOCYBE MULTICEPS *Peck*

This species of clitocybe contains an agglutinin and an haemolysin. In an extract from 4 grams to 40 cc. the agglutinin was present in dilutions of 1-10 and the corpuscles were dissolved in the mixtures containing the undiluted material and in the 1-2 dilutions. Both haemolysin and agglutinin were destroyed by heating to 60-65° C. half an hour. The

extracts produced in guinea pigs a local slough at the site of inoculation. This healed rapidly, however, and the animal recovered completely from the effects of the material. No poisonous effect upon rabbits could be determined.

The species is reported by McIlvaine to be edible and to have an oily, fishy flavor. It is regarded as edible by the Boston Mycological Club, by Atkinson (27), and by Peck. I know personally a number of individuals who are in the habit of eating it and who have experienced no ill effects in consequence.

CLITOCYBE COMPRESSIPES Peck

This species is free from haemolysin or agglutinin and has no poisonous action upon guinea pigs or rabbits. It is regarded as edible by McIlvaine.

THE LACTARII—WHITE-SPORED FUNGI WITH A MILKY JUICE.

Many of the mushrooms with a milky juice are highly prized by collectors for their edible qualities and especially for their excellent flavor. *Lactarius deliciosus* Fries, *Lactarius volemus* Fries and *Lactarius corrugis* Peck are possibly the most sought after. But few species of lactarius are regarded as poisonous to man on ingestion and of these I have had the opportunity of examining but two.

LACTARIUS TORMINOSUS Fries

Lactarius torminosus so called from the Latin "tormina" meaning "gripes" is in bad repute among mushroom eaters and is one of the few fungi with a milky juice which is clearly poisonous to man. Among the older authorities, Gillet (20) states that it is a drastic purgative, deleterious and even dangerous, and Bulliard (30) says that it is very astringent, but is eaten in Russia, preserved with salt and seasoned with oil and vinegar. Kobert (31) who attributes the species *Birkenreizker* to Schaeffer reports that it is eaten in East Russia, but is to be avoided. On the other hand, Boudier (32) calls it non-poisonous and considers that its action as a drastic purgative is no proof of its toxicity, while Cordier (33) is responsible for the statement that it is edible and that Letellier was in the habit of eating it. In this country it is regarded

by such authorities as the Boston Mycological Club as poisonous, and Atkinson (34) states that it has an acrid taste. The weight of authority seems to rest with those who regard the species as poisonous.³

The specimens of *Lactarius torminosus* submitted to me for examination contained both an haemolysin and an agglutinin. In an extract of 5 grams to 50 cc. water the haemolysin was found in a dilution of 1-33 and the agglutinin in a dilution of 1-100. The haemolysin was destroyed by heating to 60-65° C. half an hour, but the agglutinin resisted this temperature. The extracts were poisonous to guinea pigs and rabbits, in doses of 3-4 cc. In both animals the death was acute, taking place in 3-18 hours, the symptoms consisting of convulsive-like movements with retraction of the head. The picture was a little like that seen in "muscaria" intoxication, but somnolence was a more marked feature, and the typical muscaria convulsions were not seen. There were no lesions at autopsy, but the blood was fluid and seemed to have lost somewhat its coagulability. The species thus contained a poison causing an acute intoxication in small animals and this is probably identical with the substance giving rise to the gastro-intestinal disturbances in man. It was destroyed by boiling half an hour and the juice from the plant cooked in the way I have described was also free from toxicity. This is a further argument in favor of the identity of the poison found in animal inoculation, with that responsible for the symptoms following ingestion of the fungus, for Kunkel (35) states that cooking the plants and coagulating their proteid robs them of their toxicity for man, and that the cooked plant is used as food in Sweden. According to this author only the raw "torminosus" is poisonous.

LACTARIUS UVIDUS Fries

This species was free from haemolysin and agglutinin. Its heated extract was acutely poisonous to guinea pigs, 3 cc. of the usual extract killing a 400-gram guinea pig in 48 hours. There were no demonstrable lesions at autopsy. The extract had no effect upon rabbits.

Lactarius uvidus is said to be poisonous to man by Bataille (36), by Kunkel (37), and by Kobert (38). It is regarded as deleterious by the Boston Mycological Club, one of whose members has stated to me that it has an acrid taste.

³ Mr. George E. Morris tells me that the plant is on record in Vienna as having caused the deaths of two children who ate it raw and in whom medical treatment was delayed.

THE RUSSULAS—WHITE-SPORED

The majority of russulas are regarded as edible by mycologists, but two species *Russula virescens* and *Russula emetica*, are accredited with poisonous properties. The latter has a bitter taste and acts as a violent emetic. According to Kobert (39) it contains muscarin. I have examined but one species.

RUSSULA SQUALIDA Peck

This species was originally described by Peck (40) as "atro-purpurea." It was free from haemolysin and agglutinin and had an acute toxic action upon guinea pigs, 3 cc. of the extract killing a 440-gram guinea pig in three days. It had no action upon rabbits. No statement could be found in the literature as to its properties.

THE TRICHOLOMAS—WHITE-SPORED .

The tricholomas form a large genus, the members of which are sometimes said to be edible. Atkinson (41) describes but two species, *Tricholoma personatum* Fries and *Tricholoma sejunctum* Sowerby, both of which he regards as edible. I have examined one species.

TRICHOLOMA USTALE Fries

When first examined the extract from 2 grams of this fungus in 20 cc. water had no action upon blood corpuscles but 3 cc. killed a 530-gram guinea pig in three days. It was without action upon rabbits. When tested again several months later no poisonous action for either guinea pigs or rabbits could be demonstrated.

According to McIlvaine and Macadam (42), it is edible and it is also so regarded by the Boston Mycological Club, one of whose members tells me that he has tasted it with no unpleasant consequences.

THE HYGROPHORI—WHITE-SPORED

A great many different species of hygrophorus have been described, the majority being edible. They vary greatly in their dietetic value, but are highly prized by mycologists who are familiar with their properties. One species *Hygrophorus conicus* is in

bad repute, and Demange (43) has recently attributed a serious outbreak of poisoning to it.

I have examined ten different members of the group.

HYGROPHORUS PRATENSIS (*Persoon*) *Fries*

Buff Variety

This species contained no haemolysin, no agglutinin, and had no poisonous action upon guinea pigs. Regarded as edible by Cooke (44) and by the Boston Mycological Club.

HYGROPHORUS PRATENSIS

Variety "cinereus"

Probably identical with *Hygrophorus lacmus* of Kalkbrenner. It contained a small amount of haemolytic material active only in a dilution of one-half of the usual extract, but no agglutinin. Toxic to guinea pigs, 3 cc. killing a 360-gram animal in five days. Beyond a loss in weight nothing could be made out at autopsy. The extract was not poisonous to rabbits. Edible according to Peck (45) and the Boston Mycological Club.

HYGROPHORUS PRATENSIS

Variety "albus" Saccardo

Contained an agglutinin in a 1-100 dilution of an extract made from 3 grams in 30 cc. water. This agglutinin was not destroyed by heating to 60-65° C. half an hour. Contained also a small amount of blood-laking material in the undiluted extract and in the 1-2 dilutions, this haemolysin not being destroyed by heating to 65° C. The extract was poisonous to guinea pigs, 3 cc. killing a 460-gram pig in 16 days and 4 cc. killing a 590-gram animal in 25 days. Both animals developed a slough at the site of inoculation, but this rapidly healed, the animals showing, however, a slow, chronic emaciation with marked loss in weight. The lesions at autopsy were not characteristic. The extracts were not poisonous to rabbits. The species is regarded as edible by the Boston Mycological Club.

HYGROPHORUS MARGINATUS *Peck*

An extract of about 1 gram in 20 cc. of water showed an haemolysin in the undiluted material, but no agglutinin, the heated extracts being free from blood-laking action.

Three cubic centimeters produced a chronic emaciation in a 380-gram guinea pig with death at the end of 15 days; but 4 cc. of the same extract had no effect upon a 730-gram animal. The species is regarded as edible by Peck (46) and by the Boston Mycological Club. I know personally individuals who have eaten it without untoward consequences.

HYGROPHORUS HYPOTHEJUS *Fries*

An extract of 3 grams in 30 cc. contained an agglutinin in a 1-100 dilution, destroyed by heating to 60-65° C. half an hour. No haemolysin was present. A guinea pig inoculated with 3 cc. developed a progressive emaciation and died in nine days. The animal showed a broncho-pneumonia at autopsy, however, and its death could not be attributed to the inoculation. The extract had no action upon rabbits.

It is regarded as edible by McIlvaine and Macadam (47) and the Boston Mycological Club. Hollis Webster (48) states that "when dried it is crisp and nutty and very good to carry in the pocket for an occasional nibble."

HYGROPHORUS NIVEUS *Fries*

The snow-white hygrophorus

No haemolysin, no agglutinin. Not toxic to guinea pigs. This species of hygrophorus is regarded as edible by Cooke (49), by Robinson (50), and by the Boston Mycological Club.

HYGROPHORUS LAETUS (*Persoon*) *Fries*

No haemolysin, no agglutinin—non-toxic to guinea pigs. No note of the properties of this species is given in the usual literature nor by the Boston Mycological Club. I know personally, however, mycologists who are in the habit of eating it.

HYGROPHORUS PARVULUS *Peck*

But a few small pieces of this minute species could be obtained, but when these were macerated in 10 cc. of water the extract obtained was found to contain an agglutinin which was destroyed at 65° C., but no haemolysin. It was not toxic to guinea pigs.

The properties of this species are not recorded, but no bad effects follow its ingestion.

HYGROPHORUS CONICUS (*Scopoli*) Fries

An extract made from three plants sent me from the August exhibition of the Boston Mycological Club, contained an agglutinin in small quantities, but no haemolysin. This agglutinin was not destroyed by heating to 60–65° C. half an hour. The extract produced a chronic intoxication in a guinea pig of 325-gram weight, the animal dying in eleven days. It was without effect upon rabbits. Boiling the extract half an hour robbed it of its toxicity. This species is usually regarded as poisonous or suspicious, but is said to be edible by McIlvaine and Macadam (51), who state that they are in agreement with Cooke on this point. Demange, however (*l.c.*), in 1906 reported from China the poisoning of six individuals from the ingestion of fungi thought by him to be identical with *Hygrophorus conicus* of Europe. The symptoms shown by these patients consisted of violent pain located in the epigastric and umbilical region, sweating of the face and body with extreme coldness of the extremities, constipation followed by diarrhoea, and a general choleraic aspect. Of the six people affected three men died before medical attention could reach them, one woman died while being transferred to the hospital, and two men recovered after five days' treatment. The symptoms are not unlike those seen in *Amanita phalloides* intoxication. It is not impossible that the *Hygrophorus conicus* in that region differs from other members of this genus in its properties or that the plants thought to correspond to this species are a different variety.

WHITE HYGROPHORUS

A small white hygrophorus sent me by Mr. Simon Davis and regarded by him as either a new species or a variety of *Hygrophorus laetus*, was poisonous to both guinea pigs and rabbits. It contained neither haemolysins nor agglutinins. The inoculated animal died of a progressive emaciation, the guinea pig in 5 days, the rabbit in 22 days. This being the only specimen of hygrophorus showing any toxic action upon rabbits, an effort was made to confirm this finding. The same plant was therefore collected again and further studied. Its extract proved to be without action upon either rabbits or guinea pigs.

The various species of hygrophorus are thus seen to be either devoid of toxic action upon animals, or to harbor substances poisonous only to guinea pigs. The intoxication produced in

these animals is always chronic, the animals dying of a gradual emaciation with marked loss in weight. At autopsy the lesions are not characteristic.

THE ENTOLOMAS—ROSY-SPORED

The entolomas are seldom used as food and indeed are generally held in ill repute. They usually have a bad taste and are said to contain an irritant poison acting upon the mucosa of the digestive tract and causing great tenesmus, vomiting and diarrhoea, with mental and physical depression. Worthington G. Smith is known to have poisoned himself with *Entoloma sinuatum* Fries (*Entoloma fertile*), but little is known definitely as to the properties of the group. As far as could be learned no deaths have occurred from their use as food. I have examined six different species, the action of which upon animals is practically identical.

ENTOLOMA SALMONEUM Peck

An extract of 2 grams in 20 cc. water was free from haemolysin and agglutinin, but produced an acute intoxication in a guinea pig, 3 cc. killing a 390-gram pig in three days. The lymphatic glands were generally enlarged and injected, sometimes even hemorrhagic. Hemorrhagic points were found on the surface of the kidneys which were much swollen and injected. The extract had no action upon rabbits. Its edible properties are unknown.

ENTOLOMA STRICTIUS Peck

But a small quantity of this fungus could be secured. About half a gram was macerated in 8 cc. water and the extract resulting found to be without action upon blood corpuscles. It was poisonous to both guinea pigs and rabbits, 3 cc. killing a 320-gram pig in 19 days and the same amount a 1070-gram rabbit in six days. At autopsy the guinea pig showed the same swelling and injection of the glands and the punctiform ecchymoses as the guinea pig inoculated with *Entoloma salmoneum*, but the lesions in the rabbit were not characteristic. Owing to the lack of material, the toxicity of the plant could not be further tested. Its dietetic value is unknown.

ENTOLOMA CUSPIDATUM *Peck*

No haemolysin, no agglutinin. Three cubic centimeters of the usual extract killed a 300-gram guinea pig in twelve days. The pathological picture presented was much the same as that in the guinea pigs already described. The extract had no action upon rabbits. According to the Boston Mycological Club this species is edible.⁴

ENTOLOMA NIDOROSUM *Fries*

"Truffles en verte"

In an extract made from 5 grams of this fungus in 50 cc. water an agglutinin was found in a dilution of 1-100. No haemolysin was present. Three cc. of the extract killed a 265-gram guinea pig in 13 days without characteristic lesions. The same amount had no action upon rabbits. This agglutinin was at first resistant to the action of a temperature of 60-65° C. acting for half an hour. Several months later it was found in a fresh extract of the plant only in a dilution of 1-10 of an extract made from 3 grams in 30 cc. and was destroyed at 65° C. While the agglutinin had evidently deteriorated, the toxic substance was still active, 2½ cc. killing a 225-gram guinea pig in six days and 4 cc. of the extract boiled half an hour killing a 360-gram animal in 15 days. Nothing is known of its properties when eaten.

ENTOLOMA RHODOPOLIUM *Fries*

An extract made from 5 grams to 50 cc. of water was free from haemolysin and agglutinin. It was poisonous to guinea pigs, 3 cc. killing a 300-gram pig in eight days and 4 cc. killing one weighing 675 grams in 26 days. Both animals showed the characteristic swelling and injection of the glands with minute hemorrhagic areas in the internal organs. The extract had no action upon rabbits. Tested several months later the plant was again found to be poisonous to guinea pigs, but boiling the extract robbed it of its toxicity. This species of entoloma is said by Paulet to be edible, and is also so regarded by Cooke (53) and by the Boston Mycological Club.

⁴ On one occasion a rabbit weighing 1350 grams which was inoculated with 4 cc. of this extract, died acutely in about two hours. No explanation of the death of this animal could be offered. There were no manifest lesions at autopsy. Other rabbits inoculated with the same material did not show any symptoms. Similar phenomena have been noted with other fungus extracts and the possibility that the death is due to anaphalactic shock has frequently been considered.

ENTOLOMA SINUATUM *Fries*

ALSO CALLED ENTOLOMA FERTILE AND AGARICUS FERTILIS

A 35 cc. extract of about 3 grams of this fungus contained an agglutinin in a dilution of 1-10, but no haemolysin. This agglutinin was destroyed by heating to 65° C. half an hour. The extract was poisonous to guinea pigs, 3 cc. killing a 420-gram animal in twelve days. It was without action upon rabbits. Boiling the extract half an hour robbed it of its toxicity. This species is regarded by McIlvaine and Macadam (54) as probably poisonous, on the testimony of the English mycologist Worthington G. Smith, who ate an amount representing about a quarter of an ounce and nearly died from the violent illness produced. [See Stevensen (55).] The fungus is usually reported as poisonous.

THE INOCYBES—OCHRE-SPORED

The ochre-spored *inocybes* are not usually regarded as poisonous mushrooms and several species are distinctly edible. Murrill (56), however, has recently reported the poisoning of five persons from *Inocybe infida* (Peck) Earle, the specimens being collected at Forest Park, New York. The symptoms were nausea, vomiting, diarrhoea, pain and a general feeling of unrest, all the affected individuals being restored to normal health within a few hours.

Kobert (57) states that *Inocybe rimosa* Bulliard or *Hebeloma rimosum* Fries is very poisonous.

I have examined one specimen of this genus.

INOCYBE INFELIX *Peck*

Several plants of this species were sent me by Mr. Simon Davis, collected from the vicinity of Brookline, and Stow, Mass. The different plants were identical in their action. All contained both haemolysins and agglutinins. The agglutinin was present in a dilution of about 1-40 of an extract made from 5 grams in 50 cc. water and was not destroyed at a temperature of 60-65° C. maintained for half an hour. In slightly older specimens the agglutinin was only about half as strong and subsequently it showed further signs of deterioration, being less marked in its action and being destroyed at 65° C. The haemolysin occurred in much smaller quantities than the agglutinin, being found only

in the undiluted extract, but like the agglutinin it resisted a temperature of 60–65° C. Later it also deteriorated in strength. Finally specimens examined about 15 months after collection were found to be devoid of both agglutinating and haemolytic action. Extracts of this fungus were poisonous to both rabbits and guinea pigs, the intoxication coming on in a very short time, the animals showing symptoms referable to the nervous system. Thus a guinea pig weighing 550 grams, given 5 cc. of our original extract, showed a profound depression in a few minutes after the inoculation. It lay down at once in its cage, perfectly motionless and refusing to move, even under violent stimulation. It showed no convulsive movements of any description, but developed a peculiar comatose condition from which it died in five hours. The animal gave one the impression of being under the influence of a profound narcotic. At autopsy there were hemorrhages in the subcutaneous tissues about the site of inoculation, in the internal organs as the kidney and lung, while the wall of the stomach showed a number of hemorrhagic spots in the mucosa and in one area there was a distinct perforation, the contents of the stomach just beginning to escape into the peritoneal cavity. The intoxication presented by this animal was unique, similar pictures being seen with only one other fungus, *Lactarius torminosus*, which does not produce the profound narcosis, however, and with which convulsive-like movements are usually seen. Several other guinea pigs which were inoculated developed the characteristic depression, the smaller animals weighing 200 to 250 grams dying in half an hour. At autopsy the same general condition of hemorrhage was found. A rabbit given 3 cc. of the extract showed a marked effect at once. The same peculiar somnolence came on, the animal lying on its side in the cage, perfectly motionless and with retracted head. This condition lasted about five hours after which the animal began to appear more lively and again responded to stimulation. By the next morning it had completely recovered and remained perfectly well during a period of observation lasting a number of weeks. Subsequently another extract in the same proportions killed a 200-gram guinea pig over night in a 3 cc. dose, and 5 cc. killed a rabbit weighing 1380 grams in two hours, the animal showing the typical somnolence and retraction of the head. Finally 4 cc. of the extract boiled half an hour killed a 370-gram guinea pig in less than an hour.

Inocybe infelix thus contains a definite narcotic poison for both rabbits and guinea pigs, resisting desiccation and boiling. Although its toxicity for man has not thus far been reported, the poison which it

contains seems powerful and very definite in its action, and the species should certainly be avoided especially since *Inocybe infida*, a closely related form, is known to produce symptoms of illness when ingested. I have not thus far had the opportunity of examining the last named species.

Several other closely related fungi either with ochre spores or with ferrugineous spores were examined, but in no case were substances present like those in *Inocybe infelix*, the extracts being uniformly without action upon animals.

CORTINARIUS MORRISSI *Peck*

Ochre-spored.

No haemolysin. No agglutinin. Non-toxic to guinea pigs and rabbits. Properties unknown.

FLAMMULA BETULINA *Peck*

Ochre-spored

Contains an agglutinin in a dilution of 1-100 of an extract of $2\frac{1}{2}$ grams in 25 cc. water. This agglutinin was very rapid in its action and was not destroyed at 60-65° C. No haemolysin was present and the extract was not poisonous to guinea pigs. While little is known of its properties, it can be tasted without unpleasant consequences.

GALERA TENERA *Schaeffer*

Ocraceous or ocraceous-ferrugineous spores

A small quantity of this plant was sent me by Mr. Davis from the exhibit of the Boston Mycological Club. The plants were said to have caused an illness in one of the members of the Club. An extract of about a gram in 10 cc. water contained a thermo-labile haemolysin in a dilution of 1-4, but no agglutinin. The extract was without action upon guinea pigs or rabbits and there was no evidence that it contained any poisonous substance.

NAUCORIA FIRMA *Peck*

Ferrugineous spores

An extract of 4 grams in 40 cc. contained an agglutinin in a dilution of 1-10 and an haemolysin in a dilution of 1-2, the heated extracts having

the same action as the unheated. Subsequently the extract was without action upon corpuscles. It was not poisonous to guinea pigs.

No note could be found concerning its properties, but it has no bad consequences when tasted.

THE HYPHOLOMAS—PURPLE-BROWN-SPORED

A number of species of hypholoma have been described in this country, Atkinson (58) giving six or seven, two of which he considers edible, *Hypholoma appendiculatum* Bulliard and *Hypholoma sublateritium* Schaeffer. In Europe *Hypholoma sublateritium* is regarded as poisonous and is said to have a bitter taste (59). Kobert (60) states that *Hypholoma fasciculare* Hudson, the "falsche Stockschwam," is not edible while Kunkel (61) says that the same species may be poisonous, but not very, that it has a bitter taste and is thus not eaten. This species is frequently called the "sulphur top."

I have examined two hypholomas both of which produced an acute intoxication in guinea pigs, but lack of material prevented their testing on rabbits.

HYPHOLOMA INSTRATUM *Britzelmayr*

One gram of this species macerated in 10 cc. of water gave an extract free from haemolysin and agglutinin. Three cubic centimeters of this extract caused an acute intoxication in a guinea pig weighing 450 grams, the animal dying in three days. At autopsy there was an enlargement of the lymphatic glands near the site of inoculation, numerous small hemorrhages were scattered over the abdominal muscles, the mesenteric glands and the adrenals were much enlarged and small areas of hemorrhage were found on the surfaces of the kidneys and on the pleural surfaces of both lungs. A considerable portion of one lung was completely filled with blood, apparently due to the rupture of some small vessel. The same extract had no action upon rabbits. Nothing could be learned as to its properties when eaten.

HYPHOLOMA CERNUA Müller

Synonyms: Psilocybe cernua Müller; *Psilocybe cernua* (Vahl) Fries

This species in a 1 gram to 10 cc. extract contained an agglutinin in a dilution of 1-100, not destroyed by heating to 60-65° C. half an hour. No haemolysin was present. Three cubic centimeters killed a 530-gram guinea pig in four days. At autopsy a condition similar to that described above was met with. There was a dry, hard, indurated area at the site of inoculation, and minute hemorrhages in the tissues about the inguinal glands which were much swollen. The adrenals were slightly enlarged, and minute hemorrhagic points were found on the surface of the kidneys which on section were swollen and injected. The general picture was much the same as that seen in the guinea pig inoculated with *Hypholoma instratum*. This species was not tested upon rabbits. No note of its properties could be found in the literature.

PANAEOLUS RETIRUGIS Fries

Black-spored

The only black-spored agaric examined was *Panaeolus retirugis*, a species stated by McIlvaine and Macadam (62), by the Boston Mycological Club, by Atkinson (63), to be edible, but one sometimes viewed with suspicion because of its resemblance to *Panaeolus pappilloneus*, which, while edible, may produce a peculiar kind of intoxication upon ingestion. Only about 2 grams could be obtained and a 20 cc. extract of this contained neither haemolysin nor agglutinin. It was poisonous to guinea pigs, an animal weighing 325 grams dying from 3 cc. in seven days. No lesions could be found at autopsy. A rabbit weighing 1280 grams inoculated with 4 cc. died in 18 days—but was infected with coccidia. While the species may be poisonous to guinea pigs, there is no evidence of its producing an acute intoxication in rabbits and further tests should be carried out with it before a chronic intoxication can be attributed to it in these animals.

THE BOLETI—POLYPORES

The genus boletus contains some of our most valuable edible mushrooms, certain species such as *Boletus edulis* Bulliard, the "Steinpilz" of the Germans, being used in great quantities during the mushroom season. Other species such as *Boletus scaber* Fries, *Boletus granulatus* Linnaeus, are regarded as edible, but

authorities do not agree as to their flavor. The plants of this genus are usually quite large and contain a considerable amount of firm fleshy material. Were it not for the fact that some species have a bitter taste, as *Boletus Felleus* Bulliard, and others are apt to be infected with worms, as *Boletus vermiculosus* Peck, the genus would be much more valuable.

While the majority of species of boletus are edible, one or two forms are known to be very poisonous and others give rise to disagreeable symptoms, such as vomiting and diarrhoea. Thus *Boletus satanus* and *Boletus luridus* (the Hexenpilz) are everywhere recognized as poisonous species, and Kobert states that the latter form contains muscarin. Very few poisonous boleti have been reported in this country, but Collins (64) states that *Boletus miniato-olivaceus* variety *sensibilis* gathered from the Middlesex Falls Woods, produced in two individuals who ate freely of it, vomiting, purging, collapse, and a cold helpless feeling without pain, but with a narrowing of the field of vision. The symptoms came on in two hours after eating the fungi, but under the use of stimulants and strong coffee, they rapidly ameliorated and the patients eventually completely recovered.

I have examined four different species, all free from muscarin and all free from definite poisonous action.

BOLETUS CLINTONIONUS *Peck.*

Free from haemolysin and agglutinin. No poisonous action upon guinea pigs or rabbits. This fungus is reported as edible by McIlvaine and Macadam (66) and by the Boston Mycological Club, one of whose members states that he has frequently eaten the plant and that it has an insipid taste.

BOLETUS CAVIPES *Kalchbrenner*

Free from haemolysin or agglutinin—no poisonous action upon guinea pigs. Reported as edible by the Boston Mycological Club.

BOLETUS PALUSTER *Peck*

The meadow or marsh boletus

Contained an agglutinin in a dilution of 1-2 in a 20 cc. extract from about one gram. This agglutinin was destroyed at 60-65° C. No

haemolysin was present. The extract was poisonous to guinea pigs, 3 cc. killing a 430-gram pig in 17 days and the same amount killing a 345-gram pig in eight days. The extract had no action upon rabbits. Reported as edible by the Boston Mycological Club.

BOLETUS CHRYSENTERON Fries

Variety sphagnorum Peck.

Free from haemolysins, agglutinins, and poisons for guinea pigs or rabbits.

Boletus chrysenteron is regarded as edible by the Boston Mycological Club. The properties of the variety *sphagnorum* have not been reported.

MORCHELLA ESCULENTA Persoon

But one member of the group of Discomycetes or Cup Fungi was examined, *Morchella esculenta*, one of our most popular edible mushrooms. It contained neither haemolysin nor agglutinin. Its extract produced an acute intoxication in guinea pigs, a 270-gram pig dying in three days from 3 cc. and a 440-gram animal dying in six days from the same amount. The lymphatic glands were generally enlarged and injected and a few hemorrhagic areas were found on the surface of the lungs. The adrenals were much increased in size and in one instance the organ was distinctly hemorrhagic. The extract of the fungus was without action upon rabbits and when tested several months later had lost its toxicity for guinea pigs.

GENERAL CONSIDERATIONS

Haemolysins

Previous investigation has shown haemolysins to be present in *Amanita phalloides*, *Amanita virosa*, *Amanita verna* and *Amanita rubescens* in considerable quantity, while small amounts of blood-laking material were demonstrated in a species identified as *Amanita solitaria*, in *Amanita muscaria*, *Amanita frostiana* and *Amanita sprete*. Beyond the haemolysin in *Amanita phalloides*, which Abel and Ford regard as a glucoside and the hemo-lysin in *Amanita rubescens*, which Ford would class with the same substances, nothing is known as to the chemical nature of these

bodies. They were in all cases destroyed at a temperature of 60–65° C. and deteriorated rapidly in the dried fungus and in solution. The present study has confirmed our previous work on *Amanita rubescens*, *Amanita frostiana* and *Amanita sprete*, but the latter fungus has been found to contain frequently a powerful haemolysin, quite as strong as that in the “phalloides” or the “virosa.” A new species not heretofore studied, *Amanita morrissii* Peck, has also been shown to harbor a small amount of thermo-labile haemolysin.

In addition to the amanitas, haemolytic substances have been encountered in several other fungi, notably *Clitocybe multiceps*, *Lactarius torminosus*, *Hygrophorus pratensis* variety *cinereus*, *Hygrophorus pratensis* variety *albus*, *Hygrophorus marginatus*, *Inocybe infelix*, *Galera tenera* and *Naucoria firma*. In the majority of instances these haemolysins are not powerful and like those in the amanitas are destroyed at 60–65° C. *Lactarius torminosus*, however, contains a thermo-labile haemolysin in considerable amount and in *Inocybe infelix* and *Naucoria firma* the haemolysin is heat-resistant, not being destroyed at 60–65° C. Most of the fungi examined such as the entolomas, the hypholomas and the varieties of boletus seem to be free from substances dissolving the red blood corpuscles.

Agglutinins

Substances agglutinating blood corpuscles are not particularly common in plants, our knowledge of them starting from the investigations of Kobert (67) and his pupils, who first described ricin, abrin, crotin and robin. These agglutinins have served for the elucidation of a number of important problems in immunity. Similar bodies have recently been found in four species of papilionaceae by Landsteiner and Raubitscheck (68) and in six species of datura by Eisler and Portheim (69). The latter authors investigated 99 different plants and found agglutinins in but six.

Neither the agglutinins found in the papilionaceae nor those in datura are poisonous, and their introduction into animals does not cause the production of anti-agglutinins.

I have previously called attention to the agglutinin in a fungus identified as *Amanita solitaria* and to the *Muscaria-agglutinin*. The latter substance like those in the papilionaceae and in datura is not pathogenic and its injection does not cause the elaboration of antibodies. (*l.c.*)

In addition to these fungi, agglutinins have been found in thirteen other species, at least one-fourth of all the specimens examined revealing the presence of these bodies. They differed considerably in strength and showed varying sensitiveness to heat. Thus the agglutinins found in *Clitocybe multiceps*, in *Hygrophorus hypothejus*, in *Hygrophorus parvulus*, in *Entoloma sinuatum* and in *Boletus paluster* were thermo-stabile, being destroyed at 60–65° C., while the agglutinins in *Lactarius torminosus*, *Hygrophorus pratensis* variety *albus*, *Hygrophorus conicus*, *Entoloma nidorosum*, *Inocybe infelix*, *Flammula betulina*, *Naucoria firma* and *Hypholoma cernua* were all resistant to this temperature. In this respect they resembled the agglutinin in *Amanita muscara*.

The clumping of the corpuscles produced by these various agglutinins was much the same. The cells were aggregated in dense, adherent, felt-like masses which settled rapidly to the bottom of the tube and which were not broken up except with violent shaking. Rarely the clumping was less marked owing apparently to a smaller amount of agglutinating substance in the fungus, but when once the agglutination had taken place the appearances were practically identical. In many cases the agglutination of the blood corpuscles was accompanied or followed by an haemolysis. That these two phenomena were due to different substances could be demonstrated by making dilutions and by observing carefully the action of the heated extracts. Frequently an agglutination could be made out in dilutions where haemolysis was no longer visible. In but rare instances was the haemolysin resistant to a temperature of 60–65° C. and the heated extracts thus revealed a simple agglutination of the corpuscles. The haemolysins also deteriorated on standing more rapidly than did the agglutinins and old extracts were thus apt to show merely the clumping of the corpuscles. Old plants also frequently gave an extract with only an agglutinin when freshly gathered representatives of the

same species contained an haemolysin as well. In some cases, as with *Amanita muscaria*, these agglutinins were very persistent in the dried plants lasting for a number of years.

None of these agglutinins could be "activated" by the addition of lecithin or other substances like the activation of an inactivated serum-haemolysin where the heated serum simply clumps the corpuscles. Like the hemolysins in fungi the agglutinins are apparently substances acting directly upon the blood cells. Nothing has been made out in regard to the chemical nature of these bodies. That the thermo-stabile agglutinins are not of a proteid nature or at least are not coagulable proteid is evident from the fact that the solutions containing them can be heated until the coagulable proteid is thrown down, after which the filtered material is found with its agglutinative power for blood corpuscles unaltered. Like the *Muscaria-agglutinin* which these thermo-stabile agglutinins resemble, they may be glucosides or tied to some sugar containing molecule. It is well known that substances of this character are widely distributed in fungi where apparently they represent the waste products of metabolism. Both these agglutinins and those destroyed at 60-65° C. offer a fruitful field for further investigation especially from the chemical standpoint.

Poisons

The most difficult problem in the study of fungi, and from the hygienic standpoint the most important, is the determination of the toxicity of the various species for animals. This must be established by subcutaneous or intraperitoneal inoculation, feeding experiments with fungi, as a rule, giving us little information of value. Indeed there is considerable evidence to show that the *herbivora* may be entirely insusceptible to the action of the most virulent of the poisonous species when these are introduced into the stomach. Substances poisonous to animals on inoculation may, however, be quite harmless to man on ingestion, being split up into non-toxic products by the various digestive juices or for unknown reasons not being absorbed by the mucous membrane of the intestinal tract. We can not conclude therefore that spe-

cies which kill animals by our method of administration are necessarily harmful to man when eaten. Nevertheless we know that a number of fungi, such as *Amanita muscaria*, *Amanita phalloides*, *Amanita virosa* and *Amanita verna* are deadly poisonous, while other species, such as *Lactarius torminosus*, *Lactarius uvidus* and *Clitocybe illudens*, produce acute gastro-intestinal disturbances of a violent character. These forms have a definite toxicity for animals and if we compare the action of unknown species with them we may ascertain something of their properties and hazard an opinion as to the probable effect of their consumption by man.

Among the amanitas, *Amanita muscaria* has in all cases shown a convulsant action attributable to muscarin and no other species of amanita, and indeed no other species of any genera thus far examined has shown a similar activity, even the closely related *Amanita frostiana* being free from this substance. The "deadly amanita" and the poisonous varieties closely related to it, is acutely toxic to both guinea pigs and rabbits, producing fairly characteristic pathological changes which we attribute to the presence in these species of the *Amanita-toxin*. The newly examined plants, *Amanita morrissii*, *Amanita crenulata*, *Amanita citrina* and *Amanitopsis volvata* may be grouped with *Amanita phalloides*. Their extracts are in all cases poisonous to both rabbits and guinea pigs, the intoxication resembling closely that due to *Amanita phalloides*, and like the latter form, their activity is not gotten rid of by boiling with the possible exception of *Amanita citrina*. *Amanita sprete*, while it differs somewhat from the "phalloides," is acutely poisonous to guinea pigs and its boiled extract retains its toxicity. It should for the present at least be grouped with the "deadly amanita." While plants which contain traces of poisons may be eaten without harmful results, such plants under certain circumstances may develop these substances in abundance and their ingestion become a danger to man.

Amanita rubescens which has previously been shown to be free from toxicity for animals is clearly an edible species in this country and in all probability *Amanita frostiana* is of the same character. The close resemblance of the latter species to *Amanita muscaria* precludes its classification as such, however. Further

examination of the variety "formosa" of the "muscaria," which is occasionally said to possess no harmful properties for man, must be undertaken before its characteristics can be stated.

Aside from the amanitas but three species have been found which are acutely poisonous to both rabbits and guinea pigs, *Lactarius torminosus*, *Inocybe infelix* and *Entoloma strictius*. The first two species cause very peculiar intoxications in both animals, death occurring in a few hours and no pathognomonic lesions being found at autopsy. With *Lactarius torminosus* the activity of the extracts is destroyed by cooking and by boiling, the substance acting upon animals thus being almost surely identical with that producing the violent gastro-intestinal disturbances in man, since the cooked "torminosus" is innocuous on ingestion. *Inocybe infelix* has not apparently been tested thus far, in regard to its edible properties, but a closely related species, *Inocybe infida*, has been recently reported as poisonous. For the present, at least, *Inocybe infelix* should be avoided by mushroom eaters. *Entoloma strictius*, which is also poisonous to both rabbits and guinea pigs has not evidently been examined for edibility. The entolomas are in bad repute and one species, *Entoloma sinuatum*, is known to harbor a virulent poison. The other species of entoloma produce an acute intoxication in guinea pigs only, the different forms producing very similar pathological changes. It is not impossible that the toxicity of these various species may be due to a single substance enjoying a wide distribution in this genus, just as apparently a number of amanitas contain the *Amanita-toxin*.

Several other fungi such as *Lactarius uvidus* and *Clitocybe illudens*, which are known to be poisonous to man, produce an acute intoxication in guinea pigs only, having no action upon rabbits, and in two species of unknown properties, *Hypholoma instratum* and *Hypholoma cernua*, the extracts were also acutely poisonous to guinea pigs. Lack of material precluded their testing upon other animals.

The other fungi examined, somewhat over twenty in number, are either entirely devoid of poisonous action upon both rabbits and guinea pigs or produce merely a chronic intoxication in

the latter, the animals dying after the lapse of a number of days or weeks. The majority of these species are known to be edible.

From the examination of these forty species of agarics, and from our earlier studies upon twelve other species, from a study of the available literature as to their properties on ingestion, and from the evidence of a number of expert mycologists gathered by personal inquiry, we feel justified in drawing the following general conclusions as to the poisonous qualities of the species we have studied.

CONCLUSIONS

1. The fungi which are known to be poisonous to man, such as *Amanita muscaria*, *Amanita phalloides*, *Amanita verna*, *Amanita virosa*, *Lactarius torminosus*, *Lactarius uvidus*, *Clitocybe illudens*, are acutely poisonous to animals on subcutaneous inoculation, in but two instances *Lactarius uvidus* and *Clitocybe illudens* this poisonous action being limited to guinea pigs.

2. A number of other species such as *Amanita chlorinosma*, *Amanita porphyria*, *Amanita morrissii*, *Amanita sprete*, *Inocybe infelix* and others, the properties of which have not apparently been tested, resemble the poisonous forms so closely in their action upon animals as to leave little doubt that their ingestion by man is likely to be followed by serious consequences.

3. Certain genera as the entolomas and the hypholomas exhibit a uniform toxicity for guinea pigs only (except *Entoloma strictius*, which is poisonous also to rabbits). These genera include both edible and poisonous species, but no fatalities have thus far been reported from their consumption. Further experience must determine in how many of these species the poisonous substances found are harmful when taken into the intestinal tract of man.

4. The fungi described in this paper which are known to be edible are either free from action upon both rabbits and guinea pigs or produce at times a chronic intoxication in guinea pigs only.

5. A number of fungi whose properties are not described in the literature are without action upon animals. Those species are in all probability harmless to man.

6. Poisonous fungi may be divided into three groups. A. Those containing poisons acting on the nerve centers. Example, *Amanita muscaria*. B. Those producing degenerative changes in the internal organs. Examples, *Amanita phalloides*, *verna*, etc. C. Those causing gastro-intestinal disturbances of a more or less violent character. Examples, *Lactarius torminosus*, *Clitocybe illudens*, *Entoloma sinuatum*, etc.

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THE SITE OF ACTION OF STRYCHNINE IN THE SPINAL CORD¹

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INTRODUCTION

1. The site of action of strychnine in the spinal cord has been a subject of controversy. According to their conclusions those who have investigated this subject may be placed in one of three classes, *i.e.*, those who have claimed the sensory cells (or those intermediate between the posterior root ganglia and the anterior horn cells) as the site of strychnine action, those who have ascribed its action to the motor cells and those who regard strychnine as acting upon both the sensory and motor cells of the spinal cord. While the evidence in favor of the sensory action is very convincing, nothing has been done of equal weight bearing on the motor action of the drug. It has been neither proved nor disproved that strychnine acts upon the motor cells of the spinal cord.

2. We have therefore undertaken a reinvestigation of this subject in regard to both the motor and sensory cells. By the methods employed we have shown that the irritability of the sensory cells of the spinal cord is increased by strychnine in mammals, which is in agreement with previous observations on frogs. Further, we have obtained results not explicable by the view that the sole action of strychnine in the spinal cord is to increase

¹ A preliminary report of this work was given before the Society of Biological Research of the University of Pittsburgh on November 3, 1910.

² The experimental part of this work was done at the laboratory of Physiology and Pharmacology of Washington University.

the reflex irritability of the sensory or intermediate cells, this view being the one most generally held. These results seem to indicate that the irritability of the motor cells is also increased by strychnine. In the absence of such an action on the motor cells new physiological relations between the cord and motor cortex in strychnine poisoning, or different anatomical conditions in the cord than those usually held to exist must be assumed to explain the results obtained.

3. No attempt will be made to review the literature for the reason that it is voluminous and has been many times reviewed. Only such references will be made as appear of special interest in connection with our results.

4. The subject will be divided into two parts: in part I the action of strychnine on the sensory cells will be considered, and in part II the action on the motor cells.

PART I

ACTION OF STRYCHNINE ON THE SENSORY CELLS OF THE SPINAL CORD

5. Since it is impossible to anatomically isolate either the motor or sensory elements of the cord for the purpose of studying drug action, other methods must be employed. The distribution of the fibres of the sensory cells to various levels of the cord makes the physiological isolation of these cells possible in studying strychnine by applying the drug to a localized region of the cord, a method employed by Spence,³ Houghton and Muirhead,⁴

³ A. I. Spence, *Edinburgh Medical Journal*, 1866, xii, p. 44. This author applied extract of *nux vomica* to the cut upper end of the spinal cord and observed spasms in the hind legs as well as in the fore legs upon stimulation of the skin of the neck and fore legs, but only normal reflexes when the skin of the hind legs was stimulated. He concluded that strychnine acted upon the sensory or intermediate cells of the spinal cord.

⁴ Houghton and Muirhead, *Medical News*, 1895, i, p. 612. These investigators obtained results similar to those of Spence, but they extended their conclusion to exclude the action of strychnine on the motor cells.

Baglioni⁵, Filehne⁶, and probably others. After such application when symptoms of strychnine poisoning (spasms) become general, while only a limited region of the cord is poisoned, it is concluded that the poison has acted upon the sensory cells of the poisoned area. This conclusion from proper experimental evidence appears correct, but such an experiment is not designed to throw light upon the action of strychnine upon the motor cells, as will be discussed later.

6. Our experiments have been governed by the above considerations, but to make the experimental conditions more favorable we have used the method of transfusion to give the animal under observation two separate systems of circulation into one of which the drug could be injected, thus poisoning one part of the animal while the other part remained unpoisoned. The factor of anaemia, which always enters into such work on frogs is thus eliminated, the only foreign factors being the biological differences in the blood of the two animals, the short period of anaemia required to make the circulatory alteration and the peptone used to prevent coagulation, none of which have proven unfavorable. We have also used the method of applying strychnine to the exposed spinal cord in various regions. The technique of both methods is described as follows:

TECHNIQUE

Transfusion method

7. In this type of experiment the central ends of the carotid arteries and external jugular vein of a large dog, the donor, were connected to the peripheral ends of the thoracic aorta and inferior vena cava respectively of a smaller dog, the recipient, the result being that everything below the point of anastomosis in the small animal was supplied by blood from the larger animal while everything above the point of anastomosis was supplied

⁵ Baglioni, Archiv f. Physiologie, 1900, Supplement-Bd., p. 193.

⁶ Filehne, Pflügers Archiv. SS, p. 506.

by the small animal's own blood. In such an experiment a drug could be injected so that either half of the animal could be poisoned without affecting the other half while the circulatory condition of the animal as a whole remained good.⁷

8. Two animals were selected in the weight ratio of $2\frac{1}{2}$ or 3 to 1. If two operators were to work, one on each animal, both animals were etherized at the same time. Tracheal cannulae were inserted.

9. In the large animal a cannula was inserted into the femoral vein and a burette attached so that peptone and saline solutions could be given as desired. The two common carotid arteries were then dissected out up to their bifurcation and also the external jugular vein on one side. A straight cannula was inserted into each. The two carotids were then connected with rubber connections to a glass Y-tube which gave them a single outlet. Rubber connecting tubes were placed on the stem of the Y-tube and straight cannula in the external jugular vein and filled with a weak peptone solution in 0.9 per cent sodium chloride solution. Bulldog clamps were placed near the ends to prevent escape of the liquid and everything was ready for connecting to the cannulae which were later to be inserted into the thoracic vessels of the smaller animal.

10. When the above dissection was completed the burette which had been previously connected to the femoral vein was filled with warm 10 per cent peptone solution in 0.9 per cent sodium chloride solution and a slow injection of same was begun. The dose of peptone usually given was about 0.25 gram per kilo. The neck dissection should be made before the peptone is given to avoid hemorrhage. All cannulae and rubber connecting tubes were thinly coated with paraffin.

⁷ In 1907 preliminary experiments were made on the action of strychnine by a transfusion method. The transfusions were made in the abdominal aorta and vena cava below the kidneys. At that time Drs. Brooks and Guthrie were associated with us, the former being especially interested in the absorption of strychnine, while the latter was interested particularly in the technique of transfusion, suggesting the use of, and employing short hour glass cannulae to make the anastomoses. Coagulation was avoided without the use of any coagulation retarding agent.

11. After inserting the tracheal cannula in the smaller animal, the recipient, an incision was made in the mid-line of the back and a flap of skin is thrown down on one side, extending from the scapula to the lower border of the ribs. The muscles overlying the ribs were removed and six or seven ribs resected subperiosteally, being clipped near their spinal articulations with bone forceps. The intercostal arteries and muscles were ligated *en masse* by ligatures which ran from rib to rib. Artificial respiration was instituted at this stage. A flap was thrown back leaving a window in the chest wall. Four or five intercostal arteries were then ligated on each side of the aorta near their point of origin and were severed, freeing the aorta at this point from the chest wall. The thoracic duct was ligated, also the vena azygos major as it empties into the superior vena cava and again lower down in the thorax to prevent the spread of the drug from the lower to the upper part of the animal through either of these channels. The inferior vena cava was then ligated and a cannula inserted into the peripheral end. The same was done with the aorta. It is important to insert the cannula in the vein before so doing in the aorta as the period of anaemia of the spinal cord is thus reduced, for until the aorta is ligated the cord may be supplied by blood through its surrounding arterial and venous plexuses. The period of anaemia could be reduced to $2\frac{1}{2}$ minutes, but this was not done in all experiments. All cannulae and connecting tubes were filled with 0.9 per cent sodium chloride solution to displace the air, and connections were made between the carotids of the donor and aorta of the recipient and external jugular vein of the donor and inferior vena cava of the recipient. The cross circulation was then begun.

12. The strychnine was injected with a hypodermic syringe into the rubber tube connecting the arteries. The total length of the connecting tubes and cannulae was about 20 cm. The cannulae used were as large as the size of the vessels would permit. The condition of the circulation in the hind part of the small animal was determined from time to time by feeling the pulse, observing the return venous circulation and the oozing when the ball of the foot was incised.

13. When the transfusion was commenced we frequently began injecting warm 0.9 per cent sodium chloride solution into the donor through the burette and cannula in the femoral vein in which peptone had previously been injected, the amount depending upon the amount of hemorrhage, relative size of animals, etc.

14. Ligation of the inferior cava, vena azygos major and thoracic duct in the recipient obliterated the principal channels through which the strychnine might pass from the lower to the upper half of the animal. There are, however, smaller anastomosing channels which we did not attempt to obliterate by surgical methods; for example, the vessels in the spinal cord, pia and dura maters. However we do not believe that such channels offered a means of spreading the poison from the lower to the upper portion of the cord, for any flow of blood from one end of the animal to the other would probably be from the upper to the lower portion as the blood pressure in the large animal (consequently in the hind end of the small animal) was probably lower owing to the action of the peptone. Moreover, the conditions of control, we think, rule out this factor as a source of error.

15. Since the two ends of the cord in the observed animal received different blood supplies, the question of the relative state of anaesthesia must be considered. We believe that this was fairly uniform, for usually the ether was regulated with valves long before the transfusion was begun and required no further change. Here again we must look to the control conditions to preclude errors in interpretation. We believe that this has been accomplished.

METHOD OF DIRECT APPLICATION

16. In the exposure of the spinal cord in the various regions for the purpose of directly applying a drug, to obtain access to the posterior roots or to remove or sever a portion, we used only the Liston's bone forceps. However, in very large old animals we sometimes used a sharp wood chisel to remove the spines and portions of the laminae of the vertebrae to facilitate the use of the forceps. Prior to removing the bone, the muscles of the spine were removed and where hemorrhage was profuse, it was promptly

checked with hot moist towels. The hemorrhage incident to removal of portions of the vertebrae was controlled with haemostats and cotton packing. The dura was left intact and the slightest injury to the cord was avoided. When the dissection was completed the sheath was slit open. The edges of the sheath could be held open by ligatures or forceps and thus made a trough for the cord to lie in.

17. The strychnine was usually applied to the cord on cotton pads 3 or 4 cm. in length which were saturated with the poison. The trough-like arrangement of the sheath prevented the spread of the drug to the adjacent tissues.

RESULTS

18. When the lower half of the spinal cord was poisoned slowly with strychnine by the transfusion method under optimal experimental conditions symptoms of strychnine poisoning developed in the following order: 1. Exaggerated reflexes below the point of anastomosis; 2. Spasms below the point of anastomosis upon stimulation of the skin of this region by probing; 3. Spontaneous tetanus in hind part of animal (up to this point the fore legs had remained quiescent, stimulation of the skin in the poisoned area only causing spasms in the poisoned area); 4. Spasms of entire animal when lower half was subjected to cutaneous stimulation while similar cutaneous stimulation in the upper half gave only normal reflexes; 5. General spontaneous tetanus. The above steps in the march of symptoms are only the more important ones. Intermediate stages and modifying factors will be considered later. In the following protocol results obtained by the transfusion method are shown.

PROTOCOL I

STRYCHNINE ON LOWER HALF OF SPINAL CORD BY THE TRANSFUSION METHOD

*April 9, 1910**Donor: Dog A, weight 30 lbs. Recipient: Dog B, weight 16 lbs. Both females*

- 9.45 Ether to dog A.
- 10.00 Cannula in femoral vein, A.
- 10.01 Ether to dog B.
- 10.12 Began operation on thorax, B.
- 10.26 Six ribs removed, B.
- 10.35 Intercostal vessels ligated and thorax opened, B. Artificial respiration begun.
- 10.36 Another rib removed, B. Total 7 ribs.
- 10.37 Cannulae in carotids and external jugular vein and connections attached, A.
- 10.40 Began injection of peptone in femoral vein, A.
- 10.45 Vena azygos major ligated near heart.
- 10.49 Vena azygos major ligated also at a lower level.
- 10.53 Completed the injection of 35 c.c. 10 per cent peptone in A.
- 11.03 Five intercostal arteries ligated on each side at aorta and severed, B.
- 11.10 Thoracic duct incised and ligated. Lymph flowed profusely from incision.
- 11.12 Ligature around spinal column including everything except large vessels.
- 11.21 Heart beat of B, 204 per minute.
- 11.27-11.33½ Injected 150 c.c. warm 0.9 per cent sodium chloride solution in femoral vein of A.
- 11.29 Inf. vena cava ligated at heart, B.
- 11.32 Cannula inserted in vein.
- 11.32½ Aorta ligated, B.
- 11.34 Cannula inserted into aorta.
- 11.35 Arterial connection made between A and B.
- 11.36 Venous connection made between A and B.
- 11.36½ Circulation established (period of anaemia 4 minutes).
- 11.37 Pulse in femoral artery of B strong. Venous return good.

- 11.39 Hypodermic injection of $1\frac{1}{2}$ c.c. 0.5 per cent strychnine sulphate solution in A.
- 11.39-11.40 Injected 50 c.c. more warm saline into dog A.
- 11.42 Reflexes increased in A.
- 11.44 Highly exaggerated reflexes over entire body of A.
- 11.45 Slight spontaneous spasms in A. Reflexes in tail of B increased.
- 11.45 $\frac{1}{2}$ Strong spontaneous tetanic spasms of A. Artificial respiration begun.
- 11.48 Dog B as before.
- 11.48 $\frac{1}{2}$ Injected slowly $\frac{1}{2}$ c.c. 0.5 per cent solution of strychnine into arterial connection between A and B.
- 11.49 Tetanic spasm in hind legs of B, nothing in fore part of animal when skin below the anastomosis is stimulated.
- 11.50 Hind legs stiffened out in tetanic spasms. Front legs relaxed and quiescent.
- All stimuli below point of anastomosis in B give strong tetanus in hind legs, but no movement in fore part of animal. Stimulation above the point of anastomosis gives only normal reflexes. Circulation in both animals is good.
- 11.51 Muscles of posterior portion and side of neck of B exposed and the fibres of same show not the slightest contraction or twitch when the hind end of the animal is stimulated, although the hind part is thrown by such a stimulus into tetanus.
- 11.52 Injected 1 cc. more of strychnine solution into arterial connection between A and B. Immediately the hind legs of B are thrown into spontaneous tetanus which gives way to clonic spasms, while the fore legs begin to twitch.
- 11.52 $\frac{1}{2}$ Strong spontaneous tetanic spasms in fore legs, head and neck of B, while hind legs are in clonic spasms.
- 11.53 Spinal cord of dog B severed at level of the anastomosis. Spasms cease above. Ether light in B.
- 11.54 Stimulation of hind part of B gives spasms of hind legs, but none above.
- Stimulation of nose, head, shoulder or fore legs of B gives no response.
- 11.56 Same.
- 11.57 Same. Hind legs in clonic spasms.
- 11.58 Same, except that response of hind legs is weaker. Heart beat 204 per minute.
- 11.59 Heart beat of dog A, 204 per minute.

- 11.59½ Reflexes are absent in hind end of B. Cross circulation from A to B is feeble but still intact. Blood has been lost two or three times from accidental breaking of the connections.
- 12.02 Stimulation of B, both above and below anastomosis gives no response. Stimulation of head of dog A gives spasmodic twitch of head muscles alone; no response, when limbs are stimulated.
- 12.04 Transfusion interrupted. No response upon stimulating either animal.
- 12.04½ Injected intramuscularly, 1½ c.c. 0.5 per cent solution strychnine in fore part of B.
Heart beat of B, 204 per minute.
- 12.07 Reflexes slightly increased in upper part of B.
- 12.08 Repeated the injection in B.
- 12.08½ Strong tetanic spasms in upper part of B; hind end quiet.
- 12.09½ Intermittent clonic spasms in upper part of B.
- 12.11 Same, but much weaker and confined more to head and neck muscles.
- 12.13 Fore legs and shoulders now participating in weak clonic spasms, as are head and neck muscles. Reflexes still present in A.
- 12.13½ Same, but spasms stronger.
- 12.14½ Dog B, quiet.
- 12.15 Clonic spasms in B begin again, weak at first but increasing in strength.
- 12.20 Dog A, dead.
- 12.26 B continues in clonic spasms about 54 per minute. Responds to stimuli. Heart rate, 54 per minute.
- 12.35 Dog B, dead.

Autopsy on dog B

Anastomosis was made at the level of the 7th dorsal vertebrae. Inter-costal arteries from 6th to 10th inclusive were ligated. Ribs resected were 7th to 13th inclusive. Ligature around spinal column was at a level between 9th and 10th dorsal vertebrae, spinal cord was cut at the level of the 9th dorsal vertebra.

RÉSUMÉ

Period of anaemia of cord (from time aorta was ligated to beginning of transfusion) 4 minutes. Dose of strychnine sulphate, $\frac{1}{3}$ c.c. 0.5 per cent solution injected slowly into blood flowing to lower half of animal. Symptoms appeared as follows: 1. Spasms confined to lower half of body alone when skin of lower half was stimulated. 2. Spontaneous tetanic spasms in lower half of body. 3. Twitching of fore limbs begins as hind limbs were in spasms. 4. Tetanic spasms in fore part of animal and clonic spasms in lower part. The cord was then cut at the level of transfusion. Spasms immediately ceased in upper portion of animal. Stimulation in lower half of animal gave spasms in lower half, while stimulation above gave no response. Circulation in both animals was good. Strychnine was then injected intramuscularly in upper end of animal. Reflexes were first increased, followed by strong tetanic spasms in upper half of animal which later became clonic.

In the above experiment it is desired at this time to call attention to the progressive nature of the symptoms. The first symptom was a spasm of the lower part of body alone upon stimulating the skin of this region. In other experiments where the symptoms appeared more slowly, as in those in which the strychnine was applied directly to the spinal cord (see Protocols II, III and IV) this stage was preceded by one of increased reflexes in the poisoned region. So when a limited portion of the spinal cord is poisoned, all the stages of strychnine poisoning from increased reflexes to spontaneous spasms appear in the poisoned area before symptoms appear elsewhere. Later, coincident spasms occur in the unpoisoned region of the animal. In the experiment just quoted when this latter stage occurred it was shown that the upper part of the animal was not poisoned by severing the cord at the level of the points of transfusion, when the spasms ceased above but continued below the point of transfusion. A dose of strychnine in the upper part of the animal then caused the spasms to return.

19. The more gradual localized strychnine poisoning which is obtained by direct application of the drug to the exposed spinal cord is shown in the following three experiments in which strychnine was applied to the lower cervical, upper dorsal and lumbar regions of the cord.

PROTOCOL II

APPLICATION OF STRYCHNINE TO THE CERVICAL REGION OF
SPINAL CORD*December 31, 1909. Cat, 1330 grams*

- 10.20 Ether.
- 10.40 Spinal cord exposed for about 3 cm. above the upper border of the scapulae. Stimulation of the exposed cord directly with weak induced current gave contractions of muscles of neck and fore legs at different levels.
- 10.48 Sheath slit open.
- 10.48 Strychnine sulphate solution, 0.5 per cent applied to cord.
- 10.51 Reflexes much exaggerated over neck and scapulae. Stimulation here caused marked reflex contraction of neck, shoulder and fore leg muscles. Elsewhere normal.
- 10.55 Stimulation, probing of either side of neck and upper portion of scapulae is followed by a contraction of muscles of fore leg on side stimulated and a strong scratching movement of the corresponding hind leg. The scratching movement is repeated three to six times with each stimulation.
- 10.58½ More strychnine applied.
- 11.04 Same result as before except that trunk muscles now contract on side stimulated. Stimulation elsewhere is followed only by normal reflexes.
- 11.06 Conditions just obtained by stimulating skin over neck and scapulae now occur spontaneously.
- 11.12 Stimulation anywhere over head and neck is followed by a single spasmodic contraction of entire animal. Nose is especially sensitive. Stimulation elsewhere, negative.
- 11.15 Brushing fur lightly over poisoned area causes scratching movement of hind leg on same side.
- 11.18 More strychnine applied.
- 11.20 Stimulation over head, fore legs and shoulders is followed by a general spasm.
- 11.20½ General spasm on stimulating hind legs.
- 11.22 General spontaneous spasms.
- 11.24 Relaxed.
- 11.26 General spasms.

- 11.34 Weak clonic spasms. Abdominal aorta clamped and lower limbs become quiet.
Released aorta and spasms returned.
- 11.45 Animal dead.

RÉSUMÉ

Strychnine was applied to spinal cord in cervical region. The reflexes became exaggerated in this region. Stimulation of skin of either fore leg was followed by a short spasm of leg stimulated and a concomitant scratching movement of corresponding hind leg. Trunk muscles join in the spasmodic jerk when skin of the poisoned area is stimulated. Stimulation elsewhere gives only normal reflexes. Condition just described occurs spontaneously. General spasms upon stimulating skin of poisoned area. General spontaneous spasms.

PROTOCOL III

APPLICATION OF STRYCHNINE TO UPPER DORSAL REGION OF
SPINAL CORD

December 30, 1909. 1800-gram adult cat

- 11.30 Etherized.
- 12.35 Spinal cord exposed from the scapulae to the sacrum.
- 12.58 Sheath slit open in upper dorsal region and strychnine sulphate solution (0.5 per cent) applied to cord.
- 1.10 Irritating skin between upper and lower limbs causes animal to move head to side irritated.
- 1.22 Stimulation of skin over abdomen and thorax by probing is followed by a marked jerky contraction of the abdominal muscles. Stimulation elsewhere is negative.
- 1.25 Reflex as before further increased. More strychnine.
- 1.30 Same result follows stroking of fur in poisoned area.
- 1.35-1.44 More strychnine.
- 1.45 Reflexes of upper and lower limbs now increased.
- 1.47 Tetanus of trunk muscles causing back to bow out. Relaxed elsewhere.
- 1.49 Same. Abdominal muscles tense; more strychnine applied to cord.

- 1.51½ Spasm of entire animal, stronger in hind region.
1.52 Stimulation of fore limbs and head is followed by a general spasm, the hind limbs being less involved. Stimulation of hind limbs is followed only by exaggerated reflexes of these parts.
1.58 Clapping hands near ears causes general spasm.
2.06 General spontaneous convulsions. Experiment ended.

RÉSUMÉ

Strychnine was applied to the spinal cord in the upper dorsal region. Short spasmodic contractions of the abdominal muscles occurred upon stimulating skin over abdomen and thorax, which later became further increased. Normal reflexes elsewhere. This was followed by spontaneous spasms of trunk muscles. General spasms then occurred.

PROTOCOL IV

APPLICATION OF STRYCHNINE TO LUMBAR REGION OF SPINAL CORD

December 28, 1909. Grey adult cat.

Etherized. Tracheal cannula inserted. Spinal canal opened in lumbar region exposing spinal cord covered by dura.

- 11.36 Dura slit open and 0.1 per cent solution of strychnine applied.
11.38-11.48 Three more applications of 0.1 per cent strychnine.
11.51½ Application of 0.5 per cent solution of strychnine. Probing hind legs and flank is followed by jerky movement of tail.
11.56 Reflexes of lower extremities much increased. No change in upper extremities or shoulder.
12.04 Same. More strychnine applied.
12.07 Probing skin over gluteal region lightly is followed by spasms of lower half of the body. Probing fore part of animal causes only normal response.
12.17 Pia mater incised between columns of Goll and more strychnine applied.
12.24 Probing lower part of animal causes strong spasm of lower part and slight spasm of upper part.

- 12.26 Probing hind legs followed by general spasms. Probing fore legs and head produces slight increased reflexes of these regions. No response upon probing tail.
- 12.37 Spontaneous general spasms, stronger in hind than fore legs.
- 12.40 Probing fore legs causes normal reflexes.
Probing hind legs lightly causes spasm of hind legs; strongly, general spasm.
- 12.59 Stimulation of any portion of the body causes a general spasm. Sensitivity of the tail increases in passing from the tip to the root.
- 1.02 Same.
- 1.06 Strong spontaneous general convulsions.
- 1.07 Spinal cord severed in lower dorsal region.
- 1.10 Stimulation of each half of the animal causes spasm of half stimulated. Upper half tetanic and lower half clonic.
- 1.15 Same. Experiment ended.

RÉSUMÉ

Strychnine was applied to the spinal cord in the lumbar region. Stimulation of the skin of the hind legs and flank was followed first by a jerky movement of the tail and later by increased reflexes of the hind limbs. Later, stimulation of skin of hind legs was followed first by a spasm of the hind legs alone and later still by a strong spasm of the hind legs and weaker spasms of the fore legs. Only normal reflexes elsewhere. Spontaneous general spasms finally occurred.

20. So far, we have given the results of moderately slow poisoning of a limited region of the cord with strychnine by the transfusion method and still slower poisoning after local application of this drug to the exposed spinal cord. Also that the symptoms in all their stages are first local in whatsoever region of the cord the strychnine is applied. We will now give the results of rapidly poisoning a limited region of the cord. The transfusion method is most adequate for this. The results are shown in the following protocol.

PROTOCOL V

STRYCHNINE ON LOWER HALF OF SPINAL CORD BY THE
TRANSFUSION METHOD

April 2, 1910. Dog A, weight 26 lbs. Dog B, weight 11 lbs.

Arterial flow from dog A to dog B.

- 9.37 Anesthetic to dog A.
- 9.45 Anesthetic to dog B.
- 9.51 Operation completed on dog A.
- 9.58 Inserted tracheal cannula in dog B, and connected with ether bottle.
- 10.00 Began operation on chest of dog B.
- 10.05 Began injection of peptone in femoral vein of dog A.
- 10.15 Completed resection of 7 ribs of dog B.
Intercostal arteries now ligated and flap thrown back.
Artificial respiration instituted.
- 10.31 Vena azygos major ligated about 2 cm. from heart, dog B.
- 10.37 Ligation of 5 right intercostal arteries completed; severed.
- 10.47 Completed ligation of 5 left intercostal arteries and thoracic duct. Before ligating the duct an incision was made in same and lymph followed.
- 10.55-11.06 Adjusting cannulae and rubber connections on dog A.
- 11.09 Aorta dog B ligated.
- 11.10 Cannula inserted into aorta and tied.
- 11.11 Inferior vena cava ligated near heart.
- 11.12 Cannula inserted into inferior vena cava and tied.
- 11.14 Veins connected.
- 11.14½ Arteries connected.
- 11.15 Circulation opened, blood flowing well.
- 11.16 Pulse in hind legs dog B. Return circulation good.
- 11.17 Dog B relaxed, both fore and hind legs. Heart good. Pulse distinct in femoral artery. Dog A in good condition.
- 11.17-11.25 150c.c. 0.9 per cent sodium chloride solution allowed to run slowly into femoral vein of dog A.
- 11.18 1½ c.c. 0.5 per cent strychnine sulphate solution injected into tubing connecting A's carotid and B's aorta.
- 11.18½ Animal stiffening. Distinct spasms in hind legs. Stimulation over lower half of body is followed by spasms of entire animal.

- Stimulation over upper half of body gives only normal reflexes. These results were obtained three or four times.
- 11.20 Stimulation above produces no response. Stimulation below, no response.
- 11.21 Stimulation of the hind legs is followed by spasms of entire animal; the neck muscles are involved pulling the head backward or to the side; the shoulder muscles contract drawing the scapulae upward; the fore legs are not extended. Stimulation of upper part of animal, also nose, produces no response either above or below.
- 11.22 Condition same as obtained above (upon stimulation of hind legs) when any point below anastomosis is stimulated. Stimulation of upper part same as before.
- 11.24 Strong spontaneous spasms in entire animal. Spasms weaker in fore part of animal than in hind half.
- 11.25 Condition same, in response to stimulation.
- 11.27 2 c.c. more strychnine solution injected into B as before. B is in spontaneous clonic spasms, entire animal. Circulation is excellent. Jugular vein stripped of blood toward heart of A is immediately engorged.
- 11.28 Condition same.
- 11.29 Dog B quiet. Circulation strong. 50 c.c. more salt solution injected intravenously into A.
- 11.29 $\frac{1}{2}$ 2 c.c. strychnine solution injected hypodermically into dog A.
- 11.30 Stimulation of B below is followed by a very slight spasm below and a stronger spasm above.
- 11.31 Reflexes increased in dog A. Strong spontaneous spasms in B anterior to anastomosis. Began dissection of cord in mid-dorsal region of B.
- 11.32 Spasms in A.
- 11.33 Strong spasms in A.
- 11.34 Spinal cord of B severed in mid-dorsal region.
- 11.35 Blood in artery from A is dark. Eye reflexes in B active. Stimulation between scapulae, B, is followed by active twitching of fore legs. Stimulation of nose produces no response. The hind limbs are undergoing weak rhythmical contractions.
- 11.36 Artificial respiration began in dog A and continued throughout experiment.
- 11.37 Spasms in A.
- 11.39 Incision in hind leg of B bleeds freely.

- 11.39½ Stimulation of hind limbs of B gives weak clonic spasms of same but no response in upper half of animal. Stimulation over scapulae gives increased reflexes of shoulder muscles. No response to stimulation of head, neck or nose.
- 11.40 Tetanic spasms in dog A. Blood from A is bright red. Hind legs of B are perfectly quiet.
- 11.42 Spasms of A at one to two minute intervals.
- 11.43 Stimulation of skin over thighs of B is followed by a weak spasmodic contraction of hind part of animal.
- 11.45 Condition same except that spasms are stronger. Spasms in A one half to one minute intervals.
- 11.46½ Stimulation over scapulae is followed by short spasmodic contraction of corresponding shoulder muscles. No response to stimulation of neck or head except nose. Stimulation of nose causes short spasmodic contraction of all muscles above level of cord incision.
- 11.49 Same. Cross circulation almost stopped. No response to stimulation of hind end of B.
- 11.53 Stimulation of nose is followed by single contraction of neck muscles but of no others. Stimulation over scapulae causes similar contraction of corresponding fore leg. Eye reflex is good.
- 11.55½ Heart beat of B, 180 per minute.
- 12.01 Pulse weak in A; aorta clamped below kidneys.
- 12.03 Stimulation of hind limbs of A gives no response. Stimulation of fore legs is followed by slight response of corresponding limb.
- 12.04½ Clamp removed from A's aorta.
- 12.05½ Stimulation of upper portion of A gives spasms of entire animal. Stimulation of lower portion of A gives no response until level is reached at which the aorta was clamped.
- 12.07 Heart beat of B 36 per minute. Eye reflex absent.
- 12.08 A reacts the same as at last observation.
- 12.12 Both animals dead.

RÉSUMÉ

Period of anaemia of cord, 6 minutes. Dose 1½ c.c. of 0.5 per cent strychnine sulphate solution injected into blood flowing to lower half of animal. Almost immediately the hind legs were in spasms. Stimu-

lation of skin in lower half of animal was followed by general spasms. Stimulation of upper half of animal gave only normal reflexes. Spontaneous spasms of entire animal weaker in upper than in lower half. Later the spasms were stronger in upper than in lower half of animal when lower part was stimulated. Spinal cord was severed at the level of transfusion and spasms ceased above. The larger animal, A, which was used as the feeder, was given an injection of strychnine. Strong spontaneous spasms occurred. After a time the aorta was clamped just below the kidneys. After a short period of anaemia the circulation was restored. At this time stimulation of the skin above the point of occlusion caused general spasms while stimulation of the skin of the hind legs gave no response.

SUMMARY OF RESULTS

21. When the lower half of the spinal cord is poisoned with strychnine by the transfusion method spasms confined entirely to the lower half of the animal occur when the skin of that region is stimulated. The spasms then become spontaneous in the lower half of the animal.

2. As the poisoning increases the spasms become general, occurring in the upper unpoisoned half of the animal when the lower poisoned half is stimulated. Spontaneous general spasms then ensue. If at this stage the spinal cord is cut at a level just between the poisoned and unpoisoned portions the spasms cease in the upper half of the animal while they continue below, indicating that the upper half is unpoisoned. An injection of strychnine subsequently into the upper part of the animal again throws it into spasms.

3. If the poisoning be more gradual, as obtained by directly applying strychnine to the spinal cord in different regions, the symptoms of strychnine poisoning are slower to develop and stages intermediate to those just described occur, beginning with exaggerated reflexes and ending with spontaneous spasms, but always localized in the beginning to the poisoned region. Later symptoms are manifested in unpoisoned regions.

4. In an animal in a state of complete general strychnine poisoning, occlusion of the aorta was of course followed by a disappear-

ance of symptoms below the point of occlusion. After the aorta was released a time came when stimulating the skin above the point of occlusion was followed by general spasms (both upper and lower limbs) while stimulating similarly below the point of occlusion gave absolutely no response.

PART II

ACTION OF STRYCHNINE ON MOTOR CELLS OF THE SPINAL CORD

22. As previously remarked such experiments as have just been stated give no conclusive information as to the action of strychnine on motor cells of the spinal cord. While plainly indicating the action of this drug upon the sensory, or intermediate cells of the cord nothing is shown as regards the motor cells. The results which we have emphasized, regarding the localization of symptoms in the beginning, to the region poisoned, rather point to an involvement of the motor cells. Of course conclusions cannot be drawn, for it is known that the ordinary reflex paths are more easily traversed, and this no doubt, in part explains the early localization of symptoms to the area poisoned. But assuming such a motor involvement, the early symptoms of localized spasms can be explained by the combined increased irritability of the motor and sensory cells. In this stage the sensory cells have not been sufficiently poisoned to cause spasms in an unpoisoned motor region, but when their irritability is supplemented by an increased irritability of the motor cells in the poisoned area spasms occur in this region. Later when the poisoning of the sensory cells has increased we have spasms in an unpoisoned motor region and from this we get the clue that strychnine acts upon the intermediate or sensory cells. From such evidence there is no reason for concluding that strychnine does not act upon motor cells of the poisoned region of the spinal cord. The matter must be approached in a different way.

23. As shown by Baglioni⁸ and others⁹, strychnine spasms are not automatic but are reflex in nature. The tetanic spasms of

⁸ Baglioni, loc. cit.

⁹ Claude Bernard, *Leçons sur les substances toxiques*, p. 357., Poulsson. Arch. f. Exp. Path. u. Pharm., xxvi, p. 22.

strychnine poisoning are the results of sensory stimuli corresponding to the contractions of which the tetanus is composed. So even if the motor cells are rendered hyperexcitable by strychnine some impulses must reach them to set them off before we can study the nature of their condition. The adjacent sensory cells in the poisoned region of the cord cannot be made use of, for they are also poisoned and may actuate an impulse even in an unpoisoned motor cell. Van Deen¹⁰ realized this so he stimulated sensory cells in an unpoisoned region of the cord, attempting to avoid the influence of the poisoned sensory cells. He failed to consider that the unpoisoned sensory cells he stimulated were probably in communication with sensory cells of the poisoned area as well as with poisoned motor cells. The motor cells then must be reached by a path which will not affect the neighboring sensory cells. We assumed that the tract leading from the motor cortex of the cerebral hemispheres of the brain to the anterior horn cells was such a path.

24. Our plan was to first establish, for a given animal, the minimal electrical stimulus required to elicit a response in the fore and hind limbs when applied to the corresponding areas in the motor cortex, and to then poison the spinal cord in the region of the fore or hind legs with strychnine. Any difference in response to cortical stimulation of the poisoned and unpoisoned motor cells could then be observed.

25. Before proceeding we will present a few theoretical considerations which may influence the interpretation of our results. What is to be our index of poisoning of the motor cells? If we could produce a spasm involving purely poisoned motor cells and one in which only the sensory cells were poisoned, might we use the strength and duration of the spasms as a means of determining the degree of poisoning of each type of cells? The duration of the spasm is certainly not an index, for that depends upon the duration of the stimulus. The strength of the spasm involves quantitative measurements and factors with which we are not sufficiently acquainted to draw conclusions. To put the

¹⁰ Van Deen, *Physiologie de la moelle épinière*, 1860, iii, 130, (Wood's Textbook of Therapeutics).

situation in tangible form we may represent the number of motor and sensory cells in a given region of the cord each by 10. If now 1 of each type be poisoned the 1 sensory cell would be affecting practically all of the motor cells by virtue of the anatomical connections and would be registered by the action of all the 10 motor cells on the muscle, while in the case of the motor cell we would get only its single action. We merely wish to show by such an illustration that something other than differences in the strength of the spasms must be used in such comparisons.

26. We have taken then as our index of strychnine poisoning in the motor cells, changes in their irritability after strychnine poisoning as compared with the irritability beforehand or with the irritability of motor cells in unpoisoned regions of the cord, the degree of irritability being determined by the response to stimulation of the motor cortex of the brain. The question as to whether the response to cortical stimulation can be considered as an absolute index of the state of irritability of the anterior horn cells of the spinal cord is considered in the discussion.

27. The spinal canal was opened in the region to be poisoned and the skull was trephined. The following are a few remarks on technique.

TECHNIQUE

28. The exposure of the spinal cord was considered under a similar heading in the sensory portion of this paper (see par. 16). When it was desired to sever the posterior roots in the poisoned area of the cord, this was done within the dural sheath, beginning a short distance above the poisoned area and extending a short distance below.

29. The removal of the posterior half of the spinal cord when required was done as delicately as possible with a very sharp knife, resting the hand on the animal's back to avoid injury to the cord in case the animal moved.

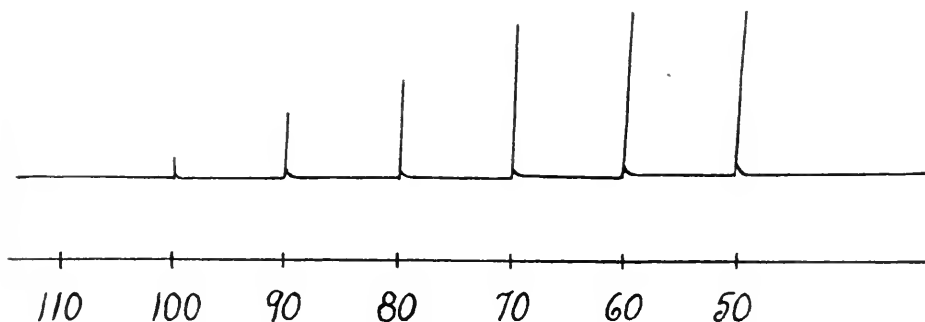
30. In exposing the motor areas of the brain the muscles were thrown back with the periosteum and a trephine was used. This opening was usually enlarged with Liston's bone forceps. In some cases the dura was left intact throughout the experiment, thus offering greater protection to the brain. Both sides of the

brain were usually exposed. We sometimes removed the entire top of the skull, dissecting away the dura with the contained longitudinal sinus, leaving the dura to cover the brain.

31. In most cases the hemorrhage from the skull was profuse, but could be stopped almost immediately by pressing a very thin layer of cotton over the bleeding bone surface for a few seconds. If, upon removal of the finger, the bleeding still continued through the layer of cotton, another thin layer was applied and this again repeated, depending upon the degree of hemorrhage. A severe hemorrhage could in this way be controlled in a very short time. The skin and muscle flaps could be joined to cover and protect the exposed brain in the intervals between observations.

32. An induced interrupted current was used, the same coil and cell being employed throughout the research. The stimulation was bipolar and throughout each experiment the distance between the poles remained constant.

33. The strength of current is expressed throughout the protocols and text in terms of millimeters distance between the primary and secondary coils. The following tracing indicates the differences in the strength of the currents we have used.



The above is a fac-simile copy, enlarged three times, of a tracing taken from the gastrocnemius muscle of a frog. It is intended to show differences in the strength of the currents which we have used in terms of response of a muscle-nerve preparation when the minimal response was obtained at 100. The figures are placed opposite the point where the sciatic nerve was stimulated and express the strength of the current in terms of distance in millimeters between the primary and secondary coils. (The above tracing was taken by Dr. C. C. Guthrie for another purpose and through his kindness we are using it.)

RESULTS

34. In five experiments the lumbar region of the cord was poisoned, and in two the cervical region was selected. The following protocol is typical of the lumbar series.

PROTOCOL VI

RESULTS FROM CORTICAL STIMULATION WHEN STRYCHNINE IS APPLIED TO THE LUMBAR REGION OF THE SPINAL CORD

May 24, 1910. Black male dog, weight 9 kilos

- 1.35 Ether.
- 1.38 Tracheal cannula inserted.
- 1.50 Began dissection of lumbar region of spinal canal from lower level of ribs to sacrum.
- 2.23 Dissection complete, cord exposed.
- 2.50 Left side of skull trephined.
- 2.55 Right side of skull trephined. More bone removed with forceps. Hemorrhage hard to control.
- 3.55 Stimulation of right motor area at 70 mm. fore and hind leg centers, causes movement of left fore and hind legs. Opposite motor area not so responsive. 70 mm. was the minimal stimulus which would bring about a response.
- 4.02 0.5 per cent strychnine sulphate solution applied on cotton to lumbar region of the spinal cord.
- 4.05 Stimulation of skin in region supplied by lumbar nerves gives increased reflexes. Reflexes normal elsewhere.
- 4.06 Stimulation of motor cortex at 70 mm. gives stronger response of hind legs than before. Response of fore legs same. Stimulation of motor cortex, hind leg area, at 80 mm. now causes a response of hind legs, but none in fore legs when fore leg area is stimulated at 80 mm.
- 4.10 Reflexes increased in hind legs. Stimulation of the motor cortex, hind leg area, at 90 mm. gives distinct contraction of hind legs. Stimulation of fore leg area with same strength current gives no response. Fore legs do not respond until current is increased to 70 mm., and then with only a slight

- twitch. Stimulation of hind leg areas at 70 mm. causes a very strong contraction of the hind legs.
- 4.17 Reflexes of both hind legs increased but greater on left side. Reflexes normal elsewhere.
- 4.19 Stimulation of right cortex: hind leg area at 100 mm. causes slight response of left hind leg; fore leg area does not cause response until current is increased to 80 mm.
- Stimulation of left cortex: hind leg area at 90 mm. causes strong response of right hind leg; fore leg area does not cause response until current is increased to 70 mm.
- 4.29 Stimulation of the left cortex, hind leg area at 90 mm. gives tetanic contractions of right hind leg. Fore leg does not respond until current is increased to 70 mm. when intermittent contractions occur. When fore leg center is stimulated at 70 mm. the hind leg also responds. The response of the hind leg is greater to cortical stimulation than to stimulation of the skin in the poisoned region.
- 5.04 Animal dead.

RÉSUMÉ

Motor areas of both sides and lumbar region of the cord were exposed. Minimal stimuli bringing about a response of both fore and hind legs on each side of cortex was 70 mm. (between coils) before strychnine was used. Strychnine was applied to the spinal cord in the lumbar region. Reflexes became increased in hind legs. The hind legs began to respond to a weaker cortical stimulus until a time came when the hind legs responded strongly at 90 mm. while the fore legs could not be made to respond until the strength of the current was increased to 70 mm. The response of the hind legs was at this time greater to cortical than to cutaneous stimulation and tetanus resulted when the stimulation of the cortex was continued while only a single twitch followed cutaneous stimulation. Stimulation of the fore leg area at 70 mm. continuously did not cause spasms but only intermittent twitchings.

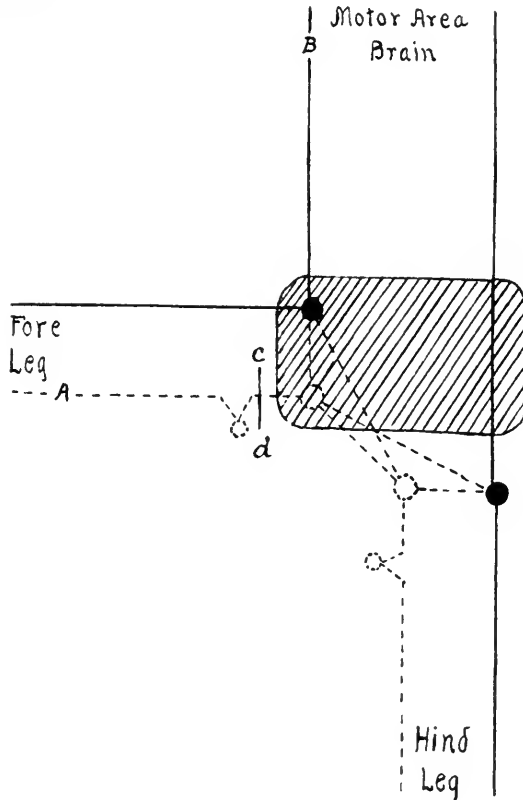
35. Instead of poisoning the spinal cord by local applications we have used the transfusion method to poison the lumbar region of the cord as described in part I of this paper. The results from cortical stimulation were similar to those obtained in the experiments where the drug was locally applied.

36. Similar results were obtained for the cervical region in two experiments when strychnine was applied to that region.

37. The results are very marked and perfectly definite and seem to indicate an increased irritability of the lower motor neurons. Current anatomical teachings would indicate this, as well as certain physiological considerations. While in the Rolandic area there are both motor and sensory elements no complication is offered by this situation as strychnine has not reached the Rolandic area and conditions there are presumably unaltered. Moreover, the control conditions seem to eliminate a possibility of error at this point, for the response of the legs in the unpoisoned region remains the same.

38. In such experiments the sensory cells of the cord are poisoned as well as the motor cells. The possible influence of the poisoned sensory cells in bringing about these results must then be considered before drawing conclusions. One question to be considered is whether the poisoned sensory cells of a given area affect the neighboring motor cells in such a way as to cause them to respond to lesser cortical stimuli. In strychnine poisoning, sensory stimuli which normally manifest no effect, are followed by reflex movements which in the early stage are confined to the stimulated area. Then it might be supposed that slight stimuli, such as pressure, air currents, etc., would cause the sensory cells to continually discharge subminimal stimuli to the motor cells of the same region, which would render the motor cells responsive to a lesser stimulus from the motor cortex. This condition would then be ruled out by repeating the experiment after severing the posterior spinal roots in the area to be poisoned. This was done and the results are shown in protocol VII which is typical of three experiments of this type. The increased excitability to cortical stimulation of the poisoned area is just as pronounced as when the posterior roots are intact.

39. As another condition, it might be supposed that the impulses from the cortex directly affected the poisoned sensory cells. In this way the increased response following cortical stimulation might be due to the influence of the sensory cells. That such an increased cortical response is not in this way due to the



Large open circles indicate sensory cells of spinal cord.

Small open circles indicate posterior root ganglia cells.

Solid circles indicate motor cells of spinal cord.

Broken lines indicate sensory paths.

Solid lines indicate motor paths.

Shaded portion indicates poisoned area of spinal cord.

When the spinal cord is poisoned with strychnine a stage is reached when stimulation at *A* causes spasms in both the fore and hind legs. When this condition is reached the posterior roots are cut at *c.d.* The motor cortex *B* is then stimulated with a current the same strength or weaker than was required to give a mere twitch of the fore leg before the strychnine was applied. If a spasm is obtained in the fore legs alone by such a stimulation it can be concluded that the sensory cells did not participate in its production, for if such had been the case the hind legs would have also been thrown into a spasm, since stimulation at *A* caused a general spasm before the posterior roots were severed. If, however, a general spasm occurred when stimulation was applied at *B* it would indicate the reverse, viz: the sensory cells had participated in producing the spasm of the fore legs.

poisoned sensory cells can be shown in the following way. If a localized portion of the spinal cord be poisoned so that cutaneous stimulation of the poisoned area causes spasms of the entire animal while stimulating the skin elsewhere is followed only by the normal reflex, and the posterior roots are then cut in the poisoned region, it can be concluded that any stimulus reaching the sensory cells from the cortical paths sufficient to cause a spasm in the muscles of the poisoned region would also cause a general spasm. In other words, if by stimulating the motor cortex in such a poisoned animal a spasm can be elicited in only the muscles supplied by nerves coming from the poisoned area while the rest of the animal remains quiet, it can be concluded that the impulses causing the spasm did not come from the poisoned sensory cells. For if such had been the case, the entire animal would have been thrown into spasms. The diagram presenting the scheme of conditions may make the proposition more easily understood.

40. In the following protocol the plan was carried out, the results indicating that the spasms following cortical stimulation are not due to impulses passing from the poisoned sensory to the motor cells. This rules out the supposed influence that the cortical impulses might have in exciting the poisoned sensory cells according to the foregoing hypothesis.

PROTOCOL VII

RESULTS FROM CORTICAL STIMULATION WHEN POSTERIOR ROOTS ARE CUT AFTER COMPLETE POISONING OF CERVICAL REGION OF THE CORD

June 2, 1910. Small dog

10.58 Ether.

11.00 Tracheal cannula inserted.

11.01 Began dissection on skull.

11.15 Motor areas located. Fore and hind legs respond at 60 mm.

11.35 Spinal cord exposed in lower cervical and upper dorsal region.

11.45 Fore leg responds slightly to cortical stimulation at 70 mm.; hind leg responds strongly.

- 11.50 Dura slit open. Large amount of fluid escaped.
- 11.54 Hind leg responds strongly to stimulation of the motor area at 70 mm. Fore leg does not respond at 70 mm. but responds moderately at 60 mm.
- 11.56 Strychnine sulphate solution 0.5 per cent applied to exposed cord and confined to dural sheath.
- 12.00 Reflexes in fore legs increased and confined to area stimulated. Stimulation of cortex at 80 mm. gives strong response of fore legs and weaker response of hind legs. Both fore legs respond when cortex on one side is stimulated while only the hind leg of the opposite side responds to cortical stimulation.
- 12.12 More strychnine applied to cord.
- 12.22 Reflexes cross over when fore legs are stimulated.
- 12.30 Short clonic spasms in fore legs lasting 1 to 2 seconds when skin of fore legs is probed.
- 12.37 Fore leg responds to cortical stimulation of 100 mm. Hind leg does not.
- 12.41 More strychnine applied to cord.
- 12.51½ Stimulation of motor cortex at 95 mm. gives tetanus of both fore legs. Hind legs do not respond until 80 mm. is reached and then only feebly and on opposite side only. Probing fore legs gives increased crossed reflex. Reflexes in hind limbs seem slightly increased at this time.
- 1.13 Same as last.
- 1.40 Pia mater gently divided between the columns of Goll and more strychnine applied.
- 1.43 Intermittent spasms of fore legs.
- 1.45 Probing skin of lower neck and shoulder causes spasms of entire animal. Repeated several times. Probing hind legs gave only normal reflexes.
- 1.53 Posterior roots severed on both sides in the region of the cord poisoned by strychnine.
- 1.59 Stimulation of the motor cortex fore leg center at 80 mm. causes tetanus in opposite fore leg and intermittent contractions of fore leg of same side, hind legs remaining quiet. Stimulation of the hind leg center at 80 mm. brings no response.
- 2.09 Stimulation of fore leg center of cortex at 75 mm. causes clonic spasms of both fore legs, greater on opposite side.
- 2.27 More strychnine applied to spinal cord.

- 2.39 Stimulation of fore leg center of motor cortex at 90 mm. gives strong tetanus of fore legs only. No response of hind legs with this stimulus.
- 2.45 Sustained tetanus of the fore leg could be obtained by stimulation of the fore leg area of the cortex at 80 mm., which did not involve the hind legs or neck muscles.
Stimulus of 70 mm. required to bring about a movement of the hind leg when its center in the cortex was stimulated.
- 2.48 Reflexes in neck muscles increased.
- 2.50½ Weak intermittent spasms of both fore legs upon stimulation of the motor cortex at 95 mm. Hind legs quiet. No response of hind legs when hind leg center is stimulated at 95 mm.
- 2.56 Few intermittent jerks of fore legs.
- 3.00 Heart rate 108 per minute.
- 3.02 Strong tetanus of fore legs and shoulder muscles when fore leg center in motor cortex is stimulated at 85 mm. Stimulation of 70 mm. required to bring about a slight movement of hind leg.
- 3.28 Strong spasm of fore legs when cortex is stimulated at 100 mm.
- 3.30 Same. Minimal stimulus causing movement of hind leg is 75 mm. Intermittent spontaneous spasmodic jerks of fore legs about 25 per minute lasting short time.
- 3.35 Reflexes of hind legs increased and cross over. Stimulation of nose and neck causes short spasmodic contraction of fore leg and neck muscles.
- 3.39 The minimal stimulus applied to the fore leg area of the cortex bringing about the slightest movement of the opposite fore leg is 110 mm. The hind leg responds to stimulation of its center at a minimum of 85 mm.
- 3.50 The left sciatic nerve exposed and stimulated at 60 mm. A contraction of both the left and right hind legs occurred, but no movement in upper part of animal.
- 4.25 Spasm of fore legs alone when cortex stimulated at 105 mm.
- 4.26 Same at 90 mm.
- 4.37 Heart rate 110.
- 4.47 Made a circular incision around the motor areas of the cortex about 1 cm. deep. Stimulation of fore leg area then gave a strong response of the fore legs at 70 mm., later at 60 mm.
- 4.49 No response could be elicited from stimulation of the fore leg area with any strength current.

- 4.53 Respiratory rate 39. Heart rate 76.
- 4.55 Injected 2 c.c. 0.5 per cent strychnine sulphate solution hypodermically.
- 5.04 Injected 1 c.c. same solution into left jugular vein.
- 5.05 Stimulation of hind legs causes general spasms.
- 5.05½ General spontaneous spasms, first tetanic and then clonic.
- 5.07½ Artificial respiration begun. •
- 5.09 Artificial respiration discontinued.
- 5.10 Clonic spasms continue at rate of about 325 per minute. They act on the thorax and abdomen in such a way as to accomplish a slight respiratory movement with each contraction, but in spite of this the alternate inspirations and expirations can be counted, being 21 per minute.
- 5.29 Spasms continue at same rate. There were two pauses of 3 seconds and another of 4 seconds, since 5.10.
- 5.39 Rate of spasms now 300 per minute.
- 5.50 Rate of spasms now 210 per minute.
- 6.09 Spontaneous spasms stopped. Probing anywhere except over fore legs gives a general spasm. Eye reflex strong.
- 6.12 Animal dead.

RÉSUMÉ

The motor areas of the cerebral cortex and the cervical region of the spinal cord were exposed. The normal response to cortical stimulation of the fore and hind legs was 70 mm., the response of the fore being a little weaker. Strychnine was then applied to the cervical region of the spinal cord. The reflexes in the fore legs became increased and stimulation of the cortex at 95 mm. gave spasms in fore legs while 80 mm. was required to bring about a weak response of the hind legs. The poisoning increased until stimulation of the skin of the shoulder produced general spasms. The posterior roots were then cut. Stimulation of the cortex after the posterior roots were cut gave spasms in the fore legs at various times from current of 100 to 80 mm., while the hind legs responded only with a twitch at 80 to 70 mm., and could not be made to respond to a weaker stimulus. After extended observations lasting several hours the animal was given strychnine hypodermically. Strong clonic spasms developed of from 325 to 200 per minute and lasted for about an hour.

41. To remove the influence of the poisoned sensory cells we have made use of two other methods, but as control conditions

were not established, the results will not be given in detail. Only brief statements of the methods will be made with such observations as may seem pertinent.

42. One method was to pare away the posterior half of the cord in either the lumbar or cervical region and to ascertain the minimal stimulus which would bring about a response in this region when the cortex was stimulated or when the cord was stimulated directly. Strychnine was then applied to the remaining anterior half of the cord and the observations repeated.

43. Another method was to poison a limited region of the cord with strychnine, the lumbar or cervical region being selected, and to follow this with applications of phenol to the same region. The response to cortical stimulation was observed before the strychnine poisoning, after applying the strychnine and following the phenol application. Briefly stated, in the one experiment performed in this manner the results were as follows: The response to cortical stimulation was increased after poisoning with strychnine, in agreement with all of our other observations. When the phenol was applied the reflexes were abolished. The response to cortical stimulation, however, still remained as strong as after the application of the strychnine alone. Phenol was suggested by the observations of Baglioni¹¹ that this drug paralyzes the sensory and stimulates the motor cells of the spinal cord.

SUMMARY OF RESULTS

44. 1. When either the lumbar or cervical region of the spinal cord is poisoned with strychnine an increase in the excitability of the poisoned area is observed when the corresponding area of the motor cortex is stimulated.

2. The same result is obtained if the posterior roots in the poisoned area are severed.

3. If strychnine be applied to the cervical region of the cord until that region is thoroughly poisoned so that stimulation of the skin of that region causes a general spasm, and then all of the

¹¹ Baglioni, loc. cit.

posterior roots are severed, it is found that stimulation of the fore leg area of the cerebral cortex will cause a spasm of the fore legs but none elsewhere. The stimulus required to bring about this result is weaker than that required in the beginning to cause a mere twitch, and is also weaker than that required to cause a twitch of the hind leg upon stimulation of its centre in the cortex at the time the cervical region is poisoned.

DISCUSSION

45. While the results obtained by cortical stimulation as reported can be explained on a basis of increased irritability of the motor cells of the spinal cord, in fact strongly suggest it, absolute proof is still wanting. There are still some hypothetical objections which must be met experimentally before such a conclusion can be drawn. Whether these hypothetical conditions are probable factors in determining results obtained is only a matter of opinion. It is our opinion that the irritability of the motor cells is increased by strychnine. We will consider, however, some of the hypothetical ways in which strychnine may act in producing the phenomena observed, in the absence of any action on the motor cells.

46. It is conceivable that the sensory cells of the poisoned region of the cord affect the motor cells of the cerebral cortex corresponding to the same region of the cord in such a way that the threshold value of the stimulus necessary to bring about a response when the cortex is stimulated, is lowered. If this were the case, it would seem that the nature of the response in the poisoned region would have been different, *i.e.*, instead of a spasmodic response which was obtained with weaker stimuli we might expect a response in muscular contractions of the intermittent type similar to the normal response, but following, of course, weaker stimuli than normal. Further, since strychnine spasms, indicating a certain degree of activity of poisoned cells are reflex, and are not developed when the posterior roots are cut, it would seem that their supposed influence in lowering the threshold value of the stimulus required to bring about a response from the

cerebral cortex would also cease when the posterior roots were cut, as was done in certain experiments. As further evidence in ruling out the influence of the poisoned sensory cells, is the experiment in which phenol was used, for this drug is said to paralyze the sensory cells. However, it is conceivable that it might at the same time increase the irritability of the motor cells offsetting the removal of the supposed sensory influence. But assuming that the poisoned sensory cells do automatically act in such a way as to affect the cerebral cortex as stated, then since we know that their anatomical connections extend to motor cells of other regions why do they not also affect the motor cells of other regions of the cord and by so acting bring about an increase in the response of other regions of the cord to cortical stimulation? We would have to assume in the absence of such action that the impulses set up in the poisoned sensory cells traveled only the paths going to the motor cortex and not those going to motor cells of the cord.

47. Or it might be assumed that intercalated cells exist in the motor path between the cerebral cortex and the anterior horn cells and that it is because of the action of strychnine on this type of cells that the increased irritability to cortical stimulation results. Whether these intercalated cells are motor or not depends upon the view point. They must in any case be conceived of as different cells than those of the cord which are the agencies through which spasms occur in parts supplied by unpoisoned regions of the cord when a poisoned region is stimulated, and which are at present largely conceived of solely as the site of strychnine action.

48. As evidence against the view that strychnine increases the irritability of the motor cells is the common observation that when a limited region of the cord in frogs is poisoned with strychnine, no increased response or spasm occurs in the poisoned region when the skin of an unpoisoned region is stimulated. Such results were obtained under the conditions of our experiments. Van Deen¹² has reported opposite results on frogs from which he

¹² Van Deen, loc. cit.

concludes that strychnine increases the irritability of the motor cells. It is possible that the condition of anesthesia in our experiments was unfavorable for the production of results similar to those of Van Deen, since it is difficult to obtain the long reflexes under anesthesia. In view of the current anatomical teachings that the sensory cells of different levels of the cord are connected, the results of Van Deen might be expected. But as previously stated they would not prove increased motor irritability. On the other hand the absence of a response in a poisoned region of the cord to stimulation of an unpoisoned region could not in view of the present anatomical conceptions be used as an argument against the view of motor poisoning, since spasms do not occur although sensory cells capable of eliciting them also exist in the same connected system. The argument would equally apply to the sensory cells. It may be possible that the sensory cells from one region of the cord do not communicate with sensory cells of another region.

49. From the above considerations our results would indicate that either the motor cells were increased in irritability; that an intercalated cell or cells existed in the path between the cortex and the anterior horn cells; or that a given region of the cord under strychnine poisoning automatically acted upon the corresponding region of the cerebral cortex in such a way as to lower the threshold value of the stimulus required to bring about a response of the anterior horn cells of the poisoned area when the cortex was stimulated.

50. Further, if the latter view be found the correct one, explaining entirely the phenomenon of increased irritability of the poisoned area of the cord to cortical stimulation, and the motor cells of the cord are not increased in irritability, then the absence of response of a poisoned region of the cord to stimulation of an unpoisoned region might be taken to indicate that the sensory elements of different levels of the cord were not directly connected.

CONCLUSIONS

51. In mammals spasms may occur under strychnine poisoning as a result of increased irritability of the sensory or intermediate cells of the spinal cord. This agrees with previous results on frogs.

52. Our results are not conclusive as regards the motor cells, but indicate that either the motor cells are increased in irritability; that an intercalated cell or cells exist in the path between the motor cortex of the cerebrum and the anterior horn cells; that a given region of the cord under strychnine poisoning automatically (as regards peripheral stimuli) acts upon the corresponding region of the cerebral cortex in such a way as to lower the threshold value of a stimulus required to bring about a response of the anterior horn cells of the poisoned area when the cortex is stimulated; or that some other action not yet understood occurs in strychnine poisoning.

Before concluding we wish to express our thanks to Drs. Stewart, Sollmann, Guthrie, Brooks and Carlson for helpful criticisms and suggestions; and to Dr. J. A. Seabold and Mr. R. H. Nicholl for assistance with some of the experiments.

THE CONTROL OF STRYCHNINE POISONING BY MEANS OF INTRATRACHEAL INSUFFLATION AND ETHER

A PRELIMINARY COMMUNICATION

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Asphyxia is the cause of death in strychnine poisoning, at least in most cases. Having this in mind Shaklee and Meltzer, about a year ago, began a series of experiments in which the availability of the method of intratracheal insufflation in strychnine poisoning was tested. The experiments were made on dogs, strychnine being administered intravenously. From a recent publication of these authors¹ the following few points may be briefly mentioned here. It was established that 0.4 mgr. strychnine per kilo body-weight is invariably a fatal dose for the dog when administered intravenously; the animals dying in less than an hour. It was further found that intratracheal insufflation alone can neither save the life of the animal nor efficiently suppress strong convulsions. The authors obtained, however, satisfactory results, when the convulsions were abolished by means of curare, while the respiration was sustained with the aid of intratracheal insufflation. In addition, the animals received intravenously, variable quantities of Ringer, to expedite the renal elimination of the strychnine as well as of the curare. Of six dogs which received 0.5 mgr. strychnine per kilo body-weight, that is, more than the minimal fatal dose, and were subsequently treated by the described method, five survived. Of twenty-two dogs which received 0.8 mgr. strychnine per kilo body-weight, that is, twice the fatal dose, thirteen animals survived the poisoning.

¹Berlin. Klin. Wochenschrift, 1910. No. 39.

We wish to report now on a series of experiments in which the convulsions were controlled by ether. These experiments were begun by Dr. Shaklee before his departure for Manila; we wish to give him herewith due credit.

We shall state our results very briefly. In twenty dogs which received intravenously 0.8 mgr. strychnine for each kilo body-weight the convulsions were controlled by means of ether, administered by intratracheal insufflation for many hours. In addition, the animals received intravenously variable quantities of Ringer. We shall not enter upon further details. *All the animals thus treated recovered completely from the strychnine poisoning and when killed later after various intervals the autopsy revealed nothing abnormal.* The average time during which the animals were treated by insufflation and ether amounts to about four and a half hours. The longest period was seven hours. During the entire procedure the animals did not seem to be in much danger, either from the effect of strychnine or from that of ether. *They did not require close watching.*

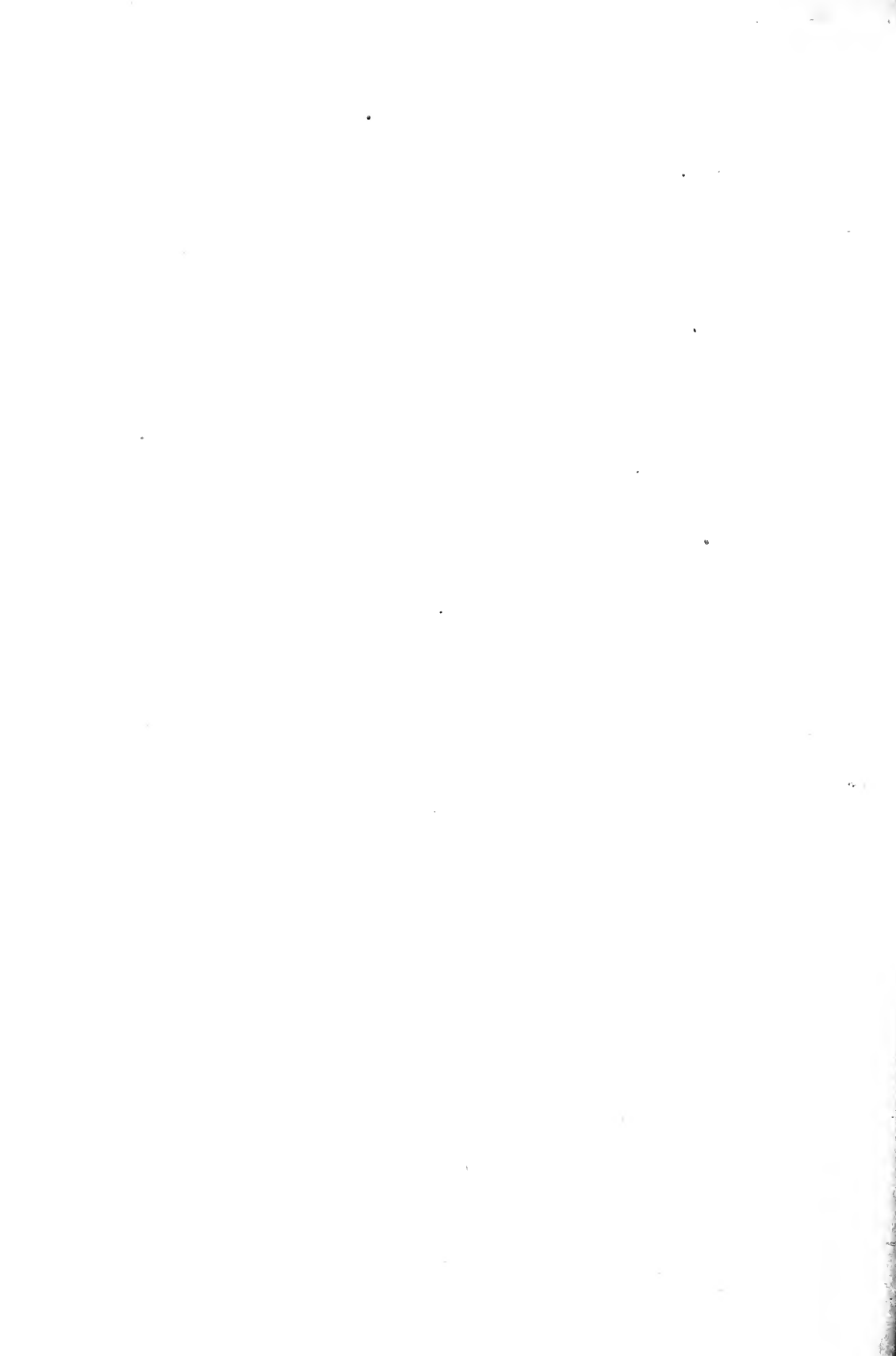
Of six dogs which received 0.8 mgr. strychnine per kilo body-weight and were treated by ether and insufflation but received no Ringer (controls), only three animals recovered from the poisoning.

In several dogs the effects of ether anesthesia alone was tested, that is, without insufflation and without the administration of Ringer. This series comprises only 9 dogs all of which received doses of strychnine exceeding the fatal one. We shall not discuss the results in detail. Of the nine dogs five succumbed. Four of these dogs received 0.8 mgr. strychnine per kilo, of which only one survived. Besides the high mortality, the plan of treating strychnine poisoning by the ordinary method of ether anesthesia is objectionable on account of the danger to which the animal is continually exposed and which necessitates the greatest attention and care. Only a degree of anesthesia which borders hard on the danger line is capable of controlling satisfactorily the effects of a fatal dose of strychnine.

We are studying also the availability of chloroform and other measures in strychnine poisoning. We are not yet prepared to

mention any details of these studies; but we may state here that according to our present experience, chloroform, even when administered by intratracheal insufflation is a much less desirable means of treating strychnine poisoning than ether.

On the basis of our experimental experience it seems to us *that the above mentioned procedure, consisting of ether anesthesia, intratracheal insufflation and intravenous infusion of Ringer's solution, offers a very effective method of treatment for strychnine poisoning in animals. We see no reason why it should not be available also for human cases.*



THE INHIBITORY ACTION OF SODIUM CHLORIDE UPON THE PHENOMENA FOLLOWING THE RE- MOVAL OF THE PARATHYROIDS IN DOGS

A PRELIMINARY COMMUNICATION

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In the course of the last two years we have made two series of experiments on dogs with intravenous injections of sodium chloride in molecular solution. We have thus become familiar with its action upon normal dogs. The first visible abnormal effect is the development of fibrillary contractions and twitchings which soon involve the muscles of the entire body. This, however, appears only after an infusion of 30 cc. or more of the solution per kilo body weight. During the foregoing stage the animal appears to be in a normal condition.

The remarkable observation of MacCallum and Voegtlin¹ with reference to the inhibitory action of calcium upon the phenomena of tetany following parathyroidectomy induced us to study the effects of the intravenous infusion of "innocuous" quantities of sodium chloride in molecular solution upon the development of these phenomena. Our problem was based upon the following considerations. The quieting effect of calcium upon the tetanic symptoms, MacCallum and Voegtlin bring into connection with the observations of J. Loeb of the inhibitory action of calcium upon the twitchings of frog muscles. They believe

¹MacCallum and Voegtlin, Jour. of Exper. Medicine, xi, 118, 1909.

that the tetany which follows the removal of the parathyroids is due to a reduction of the calcium content of the body; the tetany disappears therefore when the deficit is made good by an artificial supply of calcium. Now in Loeb's observations the twitchings of the frog muscles were developed by the action of sodium salts. Furthermore Loeb developed the conception that the irritability grows with the increase of the quotient Na/Ca . It follows from this, that, if tetany is due to a deprivation of calcium, its development ought to be hastened not only by a decrease of calcium but also by an increase of the sodium content of the body. We have then anticipated—that is, if the foregoing considerations were true—that in parathyroidectomized animals the onset and development of the tetanic symptoms would be accelerated by the intravenous infusion of an innocuous quantity of sodium chloride.

As it frequently happens in research work, the answer which our experiments impressed upon our minds most emphatically is not the one which we were looking for. In the very first experiments of this series, which were undertaken in the pretetanic stage, it dawned upon us that the quietness of the animal which often reigns during the "innocuous" period of the infusion of the solution is possibly an active phenomenon, a degree of active inhibition, which can not be manifest as long as the animal is in a quiet condition, but which may become very evident should the animal be in a hyperexcitable state. We soon began therefore to administer the infusion after the state of tetany became manifest. Our results were unmistakable. We do not intend to enter now upon particulars; this communication is only a preliminary one. We are in the midst of the investigation, and intend to do a good deal more work before attempting to offer a detailed, final presentation. As to the original problem we may indicate that under certain conditions sodium chloride may indeed act now and then by increasing the irritability of parathyroidectomized animals. What we wish to report upon now, briefly but definitely, is, however, the unmistakable inhibitory action which the infusion of sodium chloride exerts upon the symptoms following removal of the parathyroids.

The report upon our observations will be best presented, we believe, by citing some abbreviated protocols of our experiments. The inhibitory character of the action of sodium chloride in our experiments will, however, be better appreciated by comparing this action with that of calcium upon tetany. We shall, therefore, introduce the citing of our protocols by the following quotation from the protocols of MacCallum and Voegtlin on the action of calcium upon tetany.

"2107. Thyroparathyroidectomy, four days later violent twitching of muscles—pulse 160, respiration labored. Given 10 cc. of a 5 per cent solution of calcium acetate into jugular vein. Respiration became rapid, 200 to minute, twitching rare but sharp—twenty-five minutes after the injection, pulse was 80, very irregular and slow. Dog thought to be dying—occasional slight twitches. Next day dog was found walking about and fairly well but was found dead the day after."

"2207. Four parathyroids extirpated December 20. On December 22 at 3 P.M. most violent tetany, with tachypnoea, respiration 200, pulse 132, temperature 40.75. December 22 at 3:05 P.M., given 10 cc. of 5 per cent solution calcium acetate into jugular vein. 3:10, respiration 240–250, twitchings intense with snapping of teeth. 3:25, tachypnoea continues irregularly at a rate varying from 160–170. 3:30, breathing much quieter but still unnaturally rapid. No panting at present, twitching markedly improved, pulse 100. Dog now lies quietly and is fairly well relaxed. Twitchings very slight, felt only in shoulder. Lifts up his head and wanders about, is breathing quietly and seems comfortable—respiration 100, twitching has practically disappeared. 3:35, respiration 80, pulse 95—, dog now quiet and takes intelligent interest in surroundings. 3:40, respiration 35, pulse 90, slight twitching. 3:45, respiration 21, dog resting quietly—apparently rather exhausted. 3:50, runs about actively, swaying slightly with an occasional jerk of one leg, but on the whole one could not tell that he had had tetany. Responds eagerly to petting and eats greedily. 6:30, seems quite well—no trace of twitching—is very quiet and tractable, no distinct tetany.—(Continued on p. 134)— December 24 he was again found in moderate tetany, twitching very distinct, legs very stiff and as feet continually double under him, he cannot easily stand, respiration 24, labored, pulse 144, temperature 38."

¹MacCallum and Voegtlin, l. c., p. 130.

As far as we can see from the published protocols, the favorable effect of an intravenous injection of calcium acetate never lasted longer than 2 days.

We shall now proceed with the reproduction of some abbreviated protocols of our experiments.

Experiment 1. Dog. 13. November 28, 1910. Bull-terrier, fem., 5500 gms. 3:42. Etherized, intratracheal insufflation. Upper part of each thyroid removed with parathyroids.

November 30, 11:30 Shows marked symptoms of tetany, cannot stand well, lies down or falls over on side, respiration rapid, no convulsions, no grunt, marked tremor on shoulders, neck and thighs (shoulders show most). When made to walk he shuffles hind feet, back bowed up. Tied down and cannula inserted under cocaine.

11:45 Started $\frac{M}{1}$ NaCl, into left femoral vein. Respiration 180, irregular. NaCl warmed in jacketed tube, before injecting.

11:52 15 cc. NaCl in. Resp. regular and less rapid.

11:55 25 cc. NaCl in.; lies quiet, respiration regular and much slower (56 per m.). Fore legs still extended stiffly.

12:02 43 cc. NaCl; still perfectly quiet, respiration easy, regular, deeper, 32 per minute.—F. C. (fibrillary contractions) of thighs and shoulders show some diminution.

12:05 50 cc. NaCl; still quiet, F. C. definitely reduced, respiration slow, deep, regular; wide awake. All jerking motions seem abolished.

12:15 75 cc. NaCl in; respiration slow (17), regular, deep, easy. F. C., twitchings, and stiffness of fore legs, all much reduced.

12:17 83 cc. NaCl in. Stop NaCl (15 cc. per kilo). Removed from board.

12:28 He can run and even trot without sign of weakness—prefers to lie curled up in box. Unless he is examined very closely no tremor can be seen, respiration seems normal, can jump into box about one foot high using hind legs without awkwardness or difficulty.

1:50 Still lies curled up asleep in box. No tremor at all. Placed on floor he yawned, stretched and trotted over to the box and jumped in with ease—a very different appearing dog from what he was before injection.

5:00 In excellent condition. Seems to all appearances normal, walks about room, trots, takes interest in surroundings, responds to call. Ate a hearty supper, no tremor or twitchings.

December 1, 3:30 P.M. Except for very moderate tremor over thighs and shoulders he appears perfectly well and happy, runs about wagging tail, investigating boxes, etc. Doesn't seem to be losing weight. Respiration slow, easy, normal; presents a marked contrast to his appearance yesterday before injection of NaCl.

December 2, 10:00 A.M. Not in as good condition as yesterday. This is evidenced chiefly by weakness. He walks about with ease, looks well, has lost nothing in weight apparently; has a well developed moderately strong tremor.

10:12 He has had three or four attacks in the last ten minutes. He will get up and jump out of box and run about room, then suddenly he seems to feel an attack coming on and runs for box, when in, falls over on side and has a typical attack of tetany. Violent twitching. Rapid respiration, tongue hanging out. This lasts about 1 to $1\frac{1}{2}$ min- and then he will feel better and get out of box and walk around.

10:40 Tied down, cannula inserted in right femoral vein under cocaine. Rectal temperature 40.5°C .

11:00 Respiration 176 per minute, not entirely regular, strong tremors and twitching constantly.

11:00 Started $\frac{x}{y}$ NaCl into femoral (warmed in jacketed tube).

11:20 47 cc. NaCl, tremor and twitching undoubtedly diminished. An occasional twitch, tremor very moderate.

11:31 79 cc. NaCl in. Tremor very slight, takes careful inspection to see it. Twitching only slight and relatively infrequent. Temperature 39.75°C . (animal is on electric pad at medium). Lid reflex excellent.

11:39 100 cc. NaCl in; twitchings seem all gone. Respiration seems regular now, 76 per minute, temperature 39.0°C .

11:44 110 cc. NaCl in. (20 cc. NaCl per kilo). *Stopped NaCl*.

11:50 Removed from board. Respiration 17 per minute, easy, regular, deep. Walks about easily, climbed into box alone, lifted in hind legs with perfect ease, lay down in box (curled up) and began dozing. Seems normal, no tremor or twitches. A changed dog, striking contrast between conditions before and after NaCl.

12:30 Still lies curled up dozing, no tremor, an occasional single twitch of a leg, respiration 16 per minute, seems entirely normal.

December 3, 10:00 A.M. Seems in fairly good condition, appears somewhat "subdued." Prefers to lie quietly in box. Very slight tremor (which seems more like "shivering") comes with each expiration; respiration normal, slow, regular, deep; can walk about fairly easily; shows slight

weakness in climbing into box. Part of his lack of liveliness (?) seems due to discomfort from legs (where femoral cannulae were inserted).

December 4 Good condition.

December 5, 10:00 A.M. Tremor is barely perceptible, respiration is practically normal, scarcely any loss of weight, walks, trots about laboratory and jumps into box on floor without difficulty, seems in excellent condition.

December 6, 10:00 A.M. In good condition, shows interest in surroundings, respiration about normal, slow, deep, regular, no grunt. Only very moderate tremor, walks, trots about and jumps into box with ease.

December 7, 10:00 A.M. Still in good condition, slight tremor of temporal muscles, but over body practically none to be seen. Respiration slow, perhaps somewhat active. Stands, walks, trots, and jumps into box with ease. Losing weight gradually, wounds of hind legs (femoral) infected, does not eat well.

4:30 P.M. Has slept all day, didn't get out of box once; no convulsions.

December 8, 11:00 A.M. Seems improved this morning, trots across floor with ease. Shakes himself, respiration seems normal, practically no tremor at all; is getting emaciated. Wounds of legs are suppurating and eyes have become infected.

December 9, 11:00 Seems stronger than yesterday. Practically no tremor, respiration normal, no sign of convulsions for many days now. Does not eat. Becoming emaciated.

4:30 Has slept all day. He seems able to walk about with ease, jumped into box without difficulty, but walks off to find a place to sleep.

December 10 and 11. Just about the same as in the previous days. Is quite emaciated. Wounds are in bad condition.

December 12, 9:00 A.M. Found dead.

This animal developed tetany 2 days after operation. An intravenous injection of 15 cc. per kilo of an $\frac{M}{1}$ -solution of NaCl lasting about one hour changed the animal completely: respirations dropped from 170 to 17 per minute and practically all tetanic symptoms disappeared. Two days later tetany returned. This time the animal received 20 cc. per kilo of the $\frac{M}{1}$ NaCl solution in 44 minutes. The animal became strikingly changed again and all symptoms of tetany disappeared, this time never to return. The animal died 10 days after the last injection of the sodium chloride from marasmus.

Experiment 2. Dog 21, December 12, 1910, young, white male Irish terrier, 3350 grams.

12:45 Four parathyroids found and removed with upper two-thirds of each thyroid.

December 14, 9:15 A.M. In bad condition, typical tetany. Well developed tremor and twitches, respiration 216 per minute, panting; salivation. Cannot walk well, drags toes of hind feet and frequently stands on dorsum of hind feet when he puts them down in walking, staggers, and every little while falls down, occasionally rolls clear over.

9:34 Has already had two or three typical convulsions since last note. Seems in very critical condition, cannot stand now, lies on side most of time, marked tremor all the time.

10:05 Tied down and cannula inserted in right femoral, under cocaine. Respiration 252 per minute, legs stiff. Temperature 40° C.

10:11 Started NaCl $\frac{M}{8}$, femoral (warmed in jacketed tube).

10:26 100 cc. NaCl. in. Legs stiff as before. Had two convulsions since tying down; they are almost tonic in character. The tone of muscles is so great all the time, however, that the onset of a convulsion is evidenced chiefly by stoppage of respiration for a time. There is still marked tremor and twitching, respiration rapid as before. Can see no improvement as yet.

10:39 150 cc. NaCl. The convulsions are two or three per minute, or more; they are more frequent than in beginning.

10:43 Stop NaCl $\frac{M}{8}$. 165 cc. in.

10:44 Started NaCl $\frac{M}{1}$. The convulsions come so rapidly in succession as to interfere with respiration; seems in precarious condition.

10:51 17 cc. NaCl in. Respiration slow and deep with periods of stoppage, but convulsions seem to have stopped.

10:57 No convulsions for some time now. Thirty-five cc. NaCl ($\frac{M}{1}$) injected. Still considerable tremor. Respiration easy, deep, quiet, fairly regular now, 32 per minute.

11:01 A very different appearing animal, respiration excellent, regular, deep, and easy. No complaint, no twitching.

11:03 Stop NaCl $\frac{M}{1}$. 46 cc. injected. Temperature 38.3° C. No twitching or convulsions; tremor much reduced.

11:16 On floor, slightly awkward yet, but walks without much difficulty. No twitching, only slight but definite tremor. Respiration very slightly irregular but easy, deep and quiet, 32 per minute. Urinated (good quantity) when placed on floor.

11:19 Walks without staggering and without difficulty. Prefers to lie down. Very slight tremor yet, no twitches. Presents a marked contrast to his appearance before NaCl.

December 15, 10:00 In excellent condition, no one would suspect that he had ever had an attack of tetany. Walks and trots about laboratory. Comes when called. Shows no sign of tremor, twitchings or convulsions. Respiration 16 per minute, easy, regular, normal. After a time he walked off and lay down in corner.

3:00 Still lying asleep in corner. He drank some milk when it was offered to him. No sign of tremor, twitchings, or convulsions.

December 16, 10:00 A.M. In excellent condition, no tremor or twitchings. After a time, when left alone, he is found in a crouching position shows some mental depression and a slight occasional grunt.

December 17, 9:00 A.M. In better condition than yesterday! Absolutely no tremor, respiration normal, no great loss of weight yet.

December 19, 9:30 A.M. Seems about as on the 17th except there is more mental depression. Has lost some weight but not markedly. No tremor or twitching whatever. Respiration normal.

December 20, 12:00 Shows rather marked mental depression. Respiration normal, slow and regular. At times a very slight transient tremor is seen (shoulders mostly). Has lost decidedly in weight. Eats scantily.

December 21, 1:20 P.M. Presents about same appearance as yesterday. Shows moderate tremor over shoulders and thighs. Laboratory is cool (61° F.). Respiration stronger than normal, only occasionally a grunt.

December 22, Found dead.

The tetany developed two days after the operation and was violent. The attempt to relieve the tetanic symptoms with an $\frac{M}{8}$ solution of sodium chloride failed; 165 cc. had not the slightest effect, and the condition became precarious. Now the injection of 14 cc. per kilo of an $\frac{M}{1}$ solution of NaCl in 20 minutes restored the animal. The respiration which was 250 per minute before the injection became normal and the animal lost all the tetanic symptoms which never returned again. This time one injection of sodium chloride was sufficient to accomplish a permanent relief from tetany. The animal died 8 days later from exhaustion.

Experiment 3. Dog 23. December 12, 1910. Male fox terrier, 5000 grams. Four parathyroids removed; thyroids remained.

December 15, 12:30 For an hour and a half, has been having convulsions in short intervals. Lies on side, all legs extended. All the time there are very strong tremors and twitchings. Respiration rapid and labored.

1:20 Started $\frac{M}{I}$ NaCl.

1:34 50 cc. NaCl in; stop (10 cc. per kilo).

1:55 In excellent condition. Ever since being placed on floor made no attempt to lie down. No sign of a convulsion. Respiration slow, easy and regular. Presents an entirely different picture from what he did before receiving NaCl.

2:30 Eating meat; seems just like a normal dog.

December 16, 9:00 A.M. Still in good condition. Respiration normal; has a slight grunt part of time.

December 19, 11:00 A. M. Same as yesterday, grunt perhaps more prolonged.

December 20, 11:00 A.M. Shows slight tremor, slight awkwardness when walking. Only an occasional grunt. No twitching. Has lost considerable weight, eats only scantily.

December 21 Is feeling bad, seems sleepy, lying down all the time, will not eat. Grunt with each expiration. No typical tremor or twitching. Wounds of neck and leg suppurating.

December 22 Lying curled up all the time. Respiration regular and easy; no tremor, no twitching and no grunt.

December 23 Found dead.

In this animal only the parathyroids were removed and definite tetany developed three days later. An infusion of 10 cc. per kilo of $\frac{M}{I}$ NaCl sufficed to remove at once all symptoms of tetany which never returned again. The animal died 8 days after the injection from exhaustion although in this case the thyroids were left intact.

Experiment 4. Dog No. 8. Black and tan, female, 3950 grams. Excellent condition, young.

November 21, 1910. One thyroid and upper half of the other thyroid with one parathyroid, removed.

November 30 Shows nothing. Reoperated and remainder of thyroid removed.

December 5, 10:00 A.M. Well developed tremor and twitches, legs stiff. Walks with difficulty, grunts with each expiration. Respiration labored and somewhat accelerated.

2:10 P.M. Has had repeated convulsions. Getting rapidly worse.

2:15 One long continued convulsion, gasping, dying, heart still beating. Tied down and cannula inserted in femoral vein.

2:16 Only an occasional faint respiratory movement.

2:17 Started $\frac{M}{T}$ NaCl (warmed). Heart very feeble and irregular. Practically no respiration, blood blue.

2:23 40cc. NaCl in. Respiration regular, deep, easy. Heart in fairly good condition. Respiration improving rapidly.

2:26 Convulsion and retching. Moderate tremor over shoulders.

2:32 More retching. No vomitus raised.

2:38 75cc. NaCl in. Seems in good condition. Respiration very good, heart regular and strong. Excellent lid reflex.

2:41 80 cc. NaCl in. Stopped NaCl (about 20 cc. per kilo). When placed on the floor got up and walked away; does not feel very well.

3:00 Asleep. Seems in good condition. Has shown nausea twice since removal from board. Shivers slightly, especially at time of expiration.

5:00 Still asleep. No typical tremor, no convulsion since injection of NaCl. Some shivering. Roused up and placed on feet; he staggers some, grunts slightly, doesn't feel well; can walk moderately well but prefers to be quiet.

December 6, 10:00 A.M. Has a marked grunt with each expiration; only moderate tremor. Respiratory rate normal, regular and easy. Can walk or trot about without difficulty. Slight stiffness in hind legs, does not eat, no sign of convulsions.

12:00 Died.

Five days after the second operation in which the remaining part of one thyroid was removed a most acute attack of tetany developed. When the infusion of sodium chloride was started the animal was dying and apparently in a completely hopeless state. Nevertheless the infusion abolished all tetanic manifestations and the animal was restored to life. It died next day without the recurrence of evident tetanic symptoms.

Experiment 5. Dog No. 25. Black male poodle, 5000 grams. Excellent condition, young.

December 17, 1910. Left thyroid and two parathyroids from right thyroid removed.

December 19, 9:30 A.M. Shows symptoms of tetany and is in a rather critical condition. Respiration labored; stiff, walks part of time on dorsum of hind toes; lies on side with legs extended a considerable part of the time. No convulsions can be made out. Seems to lie quietly with legs extended.

1:15 Suspected pneumonia; surely not typical tetany, Lies on side almost all the time now. No convulsions have been seen; respiration labored and rather rapid. Temperature 38.5° C.

1:20 Started $\frac{M}{10}$ NaCl (warmed), right femoral vein. (This saline used by mistake.)

1:40 Stopped $\frac{M}{10}$ NaCl, 50 cc. in (10 cc. per kilo). Temperature 38.2° C.

2:00 Again lying on side. Respiration rapid and labored, no improvement.

4:30 Still on side. No improvement (just discovered the mistake in solutions above).

Started $\frac{M}{1}$ NaCl (warmed), right femoral vein.

4:47 50 cc. NaCl $\frac{M}{1}$ in. Stopped.

4:50 Walks without stiffness. Respiration much slower and easier.

4:55 Drank quite a bit of water. Has eaten nothing, since removal of parathyroids. After a short time he walked to one side of room and lay down with front paws under body, a thing he hasn't done before today.

5:45 In much better condition. Lies curled up. Respiration normal. When placed on feet could walk and trot with ease. Handles himself with ease. Holds up head easily while lying down. Presents a striking contrast to appearance before receiving NaCl. Ate heartily when placed in cage.

December 20, 10:00 A.M. In fairly good condition this morning. Can walk with ease only slight awkwardness. Gets up and approaches when spoken to. Some mental depression. Has lost considerable in weight. Very slight tremor, no twitching.

December 21, 1:30 P.M. Very emaciated. Has lain curled up most of the time since 10:00 A.M. Occasionally gets up, walks about, approaches one, wagging tail. Seems distinctly better than yesterday, no grunt or tremor or twitching. Has scarcely any difficulty in walking, but prefers to lie down. Drank milk heartily.

December 22, 11:00 A.M. In at least as good condition as yesterday, no grunt, tremor or twitching. Quite emaciated, rather weak, moderately depressed. Lies curled up nearly all the time. Doesn't respond when spoken to. Doesn't feel very well, but is in markedly better condition than before NaCl injection.

December 23, Appears about the same, no grunt. Can walk with fair ease. Lies curled up most of the time. Drank milk during morning as if he enjoyed it. No twitching or tremor, respiration normal. Very emaciated, distinct mental depression.

December 26, Lies curled up all the time. Seems in a stupor. No twitching or tremor. Cannot stand, is so weak. Doesn't eat. Tremendous cachexia, no grunt.

December 27, Found dead.

While the animal appeared to be very sick two days after the operation there were no convulsions or other well defined tetanic symptoms. The labored respiration suggested the possibility of a pneumonia. An infusion of 10 cc. per kilo of the sodium chloride solution changed the animal completely. For a few days it looked and acted like a normal animal. It died, however, 8 days after the injection, of extreme exhaustion.

These protocols will suffice to demonstrate the striking action of an intravenous infusion of an $\frac{M}{T}$ solution of NaCl upon the symptoms following the removal of the parathyroids, especially upon that group of symptoms which is designated as tetany. Almost immediately after the injection is finished the animals present a striking change and for a few days act nearly like a normal animal. The tetanic symptoms seemed to become permanently abolished by the infusion of sodium chloride. In only one instance was it necessary to repeat the infusion after two days. In practically all other cases in which a sufficient quantity of the solution was injected tetany never reappeared after the first infusion. This fact should be borne in mind when our results are compared with those of MacCallum and Voegtlin for calcium, and of Berkeley and Beebe for the nucleoproteids of the parathyroids. In either case the injections had to be repeated, the tetanic attacks usually returned a day or two after the injection. The immediate action of the infusion of sodium chloride

upon the severe tetanic manifestations is at least as good as that of calcium or of the nucleoproteids of the parathyroid glands.

All our animals which were relieved permanently of their tetany died earlier or later under signs of exhaustion. So far none of our animals have lived longer than 14 days. That interesting fact belongs to a problem which does not concern us here. We wish only to say that this has been invariably the fate of all parathyroidectomized animals whose tetanic attacks were temporarily relieved either by calcium, strontium or nucleoproteids of the glandules, many of which, however, died finally of tetany despite repeated treatment.

Against our results stands the statement of MacCallum and Voegtlin (l. c., p. 150) that "the injection of sodium (or potassium) salts has no such beneficial effect but rather tends to intensify the symptoms." We may, however, recall to mind that as a basis for this statement the authors published only one attempt with an injection of sodium acetate which the writers themselves designate (p. 136) as "especially unsatisfactory."

On the other hand MacCallum and others saw a temporary improvement from an intravenous injection of a physiological salt solution after bleeding of the animal. In these experiments, however, the essential curative action was expected to be derived from the bleeding by the removal of some of the supposed circulating toxin, the infusion of the salt solution having for its purpose only the correction of the anemia produced by the bleeding.

Regarding the nature of the remedial action of inorganic salts upon tetany MacCallum and Voegtlin assume, as stated above, that the removal of the parathyroids is followed by a deficiency of calcium in the body which is the cause of the development of tetany; hence the improvement after the artificial supply of a calcium salt. Berkeley and Beebe believe that the effect of calcium is due to its depressing property which it has in common with other members of the same chemical group, as, for instance, magnesium, strontium and even barium. Evidently neither of these theories will cover also the remedial action of sodium chloride. As to our own view, this is only a preliminary communication. We hope to be able to present more facts upon the basis of a

working hypothesis which, however, may never see light. We may add, however, that it is by no means necessary to assume that the action of various remedies which accomplish the same end are based upon a single principle.

PHYSIOLOGICAL STUDIES IN ANAPHYLAXIS: III. A MICROSCOPIC STUDY OF THE ANAPHYLACTIC LUNG OF THE GUINEA-PIG AND MOUSE

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Acute anaphylactic death in the guinea-pig has been attributed by Rosenau and Anderson (10) to asphyxia. Later Gay and Southard (8) gave a more detailed description of the respiratory phenomena observed in anaphylactic guinea-pigs. All of these writers, however, associated the cause of asphyxia with the central nervous system. It was not until the work of Auer and Lewis (3) and of Anderson and Schultz (1) working independently that the cause of sudden death in anaphylactic guinea-pigs was referred to the lungs themselves. Auer and Lewis, however, were (4) the first to publish experimental data on the subject and inferred from their experiments that the asphyxia is due to an inspiratory immobilization of the lungs caused by a tetanic contraction of the smooth musculature of the bronchioles and alveolar ducts, thus preventing ventilation of the alveolæ. In a subsequent article these investigators (4) again speak of the point of contraction and occlusion as located in the bronchioles (p. 165). In a more recent paper, Auer (5) ascribes the asphyxia of serum anaphylaxis to a "tetanic contraction of the *bronchial muscle*, the contraction being so pronounced that the lumina of the *smaller bronchial tubes* (italics our own) were occluded, thus preventing both entrance and escape of air" (p. 638). The anatomical or experimental evidence for this apparent shift in the location of the point of constriction does not appear. Indeed, in view of the earlier hypothesis which he shared with Lewis—and which was adopted by a number of other workers in this field—and more especially, the fact that he makes no specific mention of a change

of opinion, it seems probable that Auer uses "smaller bronchial tubes" and "bronchioles" synonymously. However this may be, the prevailing idea seems to be that the muscular constriction causing death by asphyxia in hypersensitized guinea-pigs is located somewhere in the terminal tubules of the bronchial tree (bronchioles).

Biedl and Kraus (6), by rather crude methods, come to the conclusion that the air passages were obstructed by a folding of the mucous membrane in the "Bronchien"; they admit that by their histological methods they could not say positively that the folding was due to contraction of the smooth muscle since the same condition was found in normal lungs of pigs dying from asphyxia, the chief difference being that in the normal lung the alveolæ were less inflated, the walls being thicker and traversed by blood vessels containing blood. No cuts or accurate descriptions of sections are given. The term "Bronchien" is used loosely and since the same term is used in referring to the structures indicated in Auer and Lewis' first paper it is evident that the bronchioles and possibly the pre-bronchioles or larger bronchioles containing mucous membrane are meant.

Biedl and Kraus (6) in describing the anaphylactic phenomena observed in dogs state that convulsive expiratory phenomena are seen but at no time is marked dyspnoea present. Arthus (2) thought he observed an acceleration of the respiratory movements, but did not study it any more carefully than did Biedl and Kraus. Arthus (2) also studied the rabbit and states that at the time of the fall of blood pressure the respiratory rate is increased leading to a state of polypnoea. Friedberger and Hartoch (7) also noticed a change in the character of the respiration that accompanied the fall of blood pressure in rabbits. The results obtained by these various workers on the cat, dog, etc., *i. e.*, absence of dyspnoeic symptoms at the second injection of serum, and the peculiar human reactions observed in serum-therapy, call for a careful comparative microscopic study of the lungs of the animals concerned.

White mice, probably because of the absence of sudden death from asphyxia, have been classed by several writers as animals incapable of anaphylaxis. For the present it may suffice to note

that white mice do react towards horse serum. Non-sensitized mice like non-sensitized guinea-pigs are killed by sterile horse serum injected into the jugular vein. The dose, however, is larger than that necessary to kill similar animals previously sensitized. White mice may also die after injecting 2 to 4 cc. of sterile serum into the abdominal cavity. The death rate in sensitized mice may be slightly higher than that for normal mice, but out of the large number of mice used in these experiments none died suddenly from asphyxia. The symptoms observed in both sensitized and normal mice after injecting 2 cc. of serum are qualitatively very much alike, differing, however, somewhat quantitatively in their action. After injecting a second dose, from $\frac{1}{2}$ to 5 cc., of serum there is a short period of excitement in which the mouse may show marked irritability of the skin and rapid respiration; the animal scratches and licks itself vigorously, passes urine and feces, examines its abdomen and seems very restless. A period of depression then follows; there is a fall of blood pressure and of temperature, and slowed and forced respiration. The respiration finally becomes very slow and shallow at which time the mouse lies limp, unable to walk or to stand up, and vigorous pinching is unnoticed. In this condition the mouse may die, or recover completely, or it may partly recover and die some days later. In some of the sensitized mice that recover there is a shriveling and drying off of the ears, a thinning out of the hair, the skin being reddish in spots as if diseased.

The lungs of mice that die from the second injection resemble those from normal mice killed with serum or by other means. They are collapsed, but often one finds in the various lobes red liver-like areas; or the lungs are reddish, as contrasted with the pink color of a recently killed mouse. The right heart of mice dying from serum is, as a rule, filled with blood and the veins are congested. Low temperature favors an increased death rate; this is what one might expect if death results primarily from low blood pressure.

Our results with white mice and with cats mark the immediate starting point for the comparative histological study here to be detailed. The present paper deals primarily with the compara-

tive anatomy of the anaphylactic and normal lungs of the guinea-pig. The anatomy of the lung of the mouse as compared with that of the guinea-pig explains at once why the sensitized white mouse does not show fatal respiratory symptoms at the second injection of serum. Further studies aim to extend the histologic observations made on the guinea-pig and mouse to other animals, especially the cat, dog, rabbit, and man.

The lungs used in these experiments, unless otherwise stated, were removed and fixed a few minutes after death. Large quantities of fresh fixing fluid were used. For every lung of a sensitized animal used there was a normal control, the animal being killed by crushing the medulla (in a few cases the trachea was pinched off). The trachea of some specimens was tied off before opening the chest; in others not, the lungs and trachea then being removed and fixed. In other experiments the fixing solution was first injected into the pulmonary artery and then the lungs cut into small pieces and fixed or the lungs were fixed *in toto*. After experimenting with a number of fixing fluids, Carnoy's strong solution was found most satisfactory. Sections were cut in paraffin and stained with Delafield's hæmatoxylin and picro-acid-fuchsin. By this method the fibrous connective tissue was clearly differentiated from the smooth muscle. It was at once seen that if the contraction and occlusion of the bronchioles (supposed cause of asphyxia) in the guinea-pig were due solely to the *amount* of smooth muscle, then the mouse should show the same anaphylactic symptoms at the second injection of serum if properly timed as to sensitization. For, while the whole plan of structure in the lung of the mouse as compared with that of the guinea-pig is less coarse, yet the more proximal portion of the bronchial tree in the former (as regards the amount of smooth muscle) is very similar to the more peripheral (bronchioles) portion of that of the latter. And it seemed a matter of no importance from the point of view of the final result as to whether the occlusion was proximal or distal—in fact in the former case the occlusion would appear to be more effective in producing asphyxia. Consequently, other possible factors besides the quantity of smooth muscle (the maximum

contraction of which is probably a fairly definite quantity) had to be searched for.

The study of the anaphylactic lung of the guinea-pig was begun at the tips of the several lobes (c. f. fig. 1 for the anatomy of the guinea-pig lung) and carried towards the attached point. It was at once discovered that the bronchioles and alveolar ducts were *wide-open*—in fact the whole peripheral portion, including the

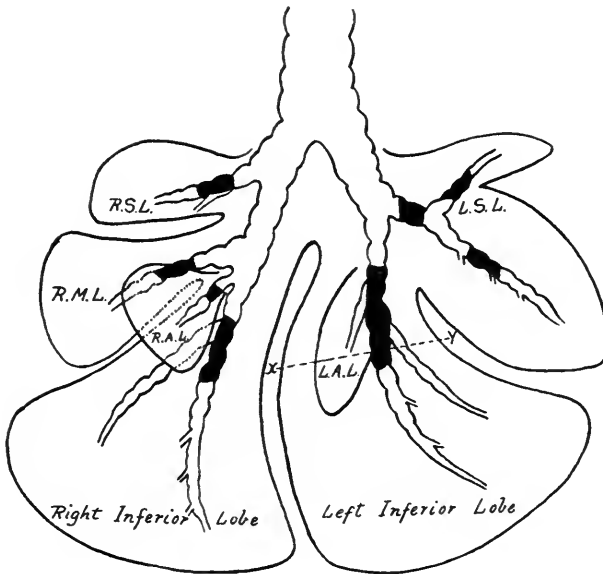


FIG. 1. Diagram of anaphylactic lung (ventral view) of guinea-pig, showing the levels of stenosis (black) in the bronchial tree. R. S. L. and L. S. L. = Right and left superior lobes; R. M. L. = Right middle lobe; R. A. L. and L. A. L. = Right and left accessory lobes.

alveolæ, appeared distended. No complete occlusion was at first discovered until the level of the secondary bronchi was reached and at a point in these, close to the level where they leave the primary bronchi (fig. 2). Here, the lumen was obliterated by apposition and interlocking of the folds of the mucosa and the included larger or smaller mass of mucus. The considerable amount of smooth muscle was contracted, carrying the mucosa away from the pe-



FIG. 2. Photomicrograph of section of anaphylactic lung at approximately the level *x-y*, fig. 1. The medium-sized bronchus to the right of the larger closed bronchus is also practically occluded. To the left is the pulmonary artery showing a slight segmentation of its musculature. Above is a bronchiole, *wide open*. The alveoli are distended. The connective tissue between the muscle of the bronchus and the cartilage plates is oedematous and congested.

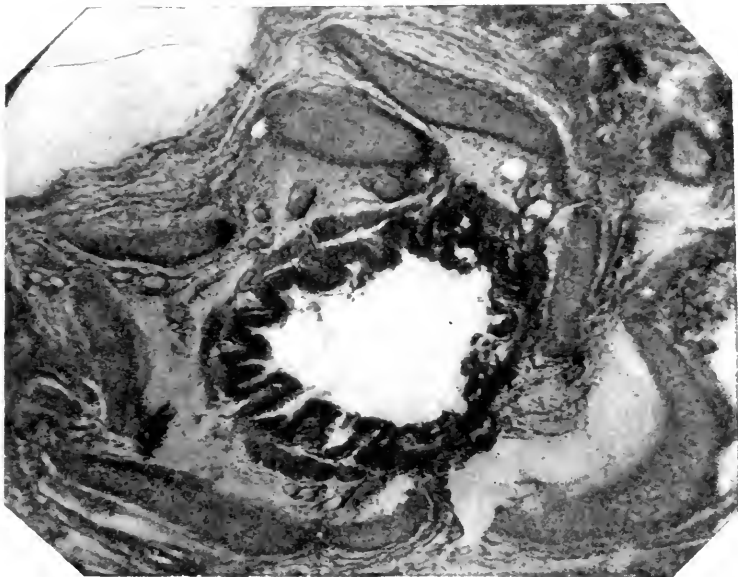


FIG. 3. Photomicrograph of cross section of secondary bronchus of the normal lung at a level slightly above $x-y$, fig. 1. The spaces about the right half of the bronchus are artifacts produced in sectioning. The mucosa is greatly folded, but the lumen is wide open.



FIG. 4. Photomicrograph of oblique cross section (mucosa broken below) of bronchus of normal lung at about the level $x-y$, fig. 1, the mucosa is very greatly folded (accentuated by action of fixing fluid) but a distinct lumen is maintained. A cross section of the pulmonary artery through a muscle segment is shown above to right.

ripheral cartilage plates. A further indication that the folding of the mucosa is actually due to contraction of the smooth muscle is the character of the nuclei, *i. e.*, short, stout, with peripheral net knots, and frequently curled—but no contraction bands very conspicuous in the cytoplasm. The intervening layer of connective tissue was oedematous and congested. A study of the bronchi of normal lungs at this level reveals that normally the mucosa here is greatly folded (fig. 3) and that the lumen, relative to the wall of the tube, is quite small. The picture of the preserved system is probably not absolutely true to life (since preserving fluids cause a certain amount of contraction of the smooth muscle) but the difference between the bronchial tubes at this level in the normal and the anaphylactic guinea-pig lung, while not great, is specific and very significant. The difference is one between small lumen (probably constricted—due to action of fixing fluid) and occluded lumen. Since the maximum amount of contraction is probably fairly constant, the smooth muscle of the anaphylactic lung, already contracted by the second injection of serum, was so fixed at death and probably not further contracted by Carnoy's fluid.

In the anaphylactic lung the oedema and congestion are not limited to the occluded portions of the tubes. The connective tissue around the bronchial tubes is everywhere oedematous and usually all the capillaries and small veins are gorged with blood. In the normal lung both congestion and oedema are absent and the alveoli are small, with thicker (contracted) walls and often collapsed. Moreover, the bronchioles and smaller bronchi also appear collapsed, thus showing a very narrow lumen. In the anaphylactic lung, on the other hand, these same tubes appear distended (fig. 2). The interpretation seems justified that during anaphylactic shock inspiration continues for several breaths unaccompanied by expiration. This interpretation appears the more reasonable in view of the fact that in the laboratory guinea-pigs the expiratory muscles are only moderately developed. It would seem, then, that in the bronchioles the expansive force of the inspired air is greater than the combined contracting energy set loose by the serum and the fixing fluid. Macroscopically, the anaphylactic lung is in bulk approxi-

mately twofold that of the normal. The corresponding microscopic conditions are marked by extreme inflation of the alveoli and widespread collapse in the anaphylactic and normal lungs respectively.

It remained to locate definitely the level or levels (and their limits) of the areas of occlusion. This was done by preparing complete dissections of the bronchial tree of both types of lungs, clearing the same in oil and making toto mounts in balsam. Even macroscopically the difference between the two systems is striking, and the level of occlusion can be vaguely discerned. With the binocular microscope, the limits of constriction can be definitely marked. Fig. 1 illustrates the findings. It will be noted in general that the point of occlusion is just beyond the place where the secondary bronchi leave the primary, and in all cases at points commanding large areas of lung tissue. In fact the occlusion is at the very point where it can most effectually produce asphyxia. These anatomical results accord best with the condition of very rapid collapse (2 to 5 minutes) observed in succumbing guinea-pigs. The limits of the areas of occlusion probably vary somewhat in different individuals and it must be noted that the occlusion is not quite total throughout the entire area of stenosis. There are levels in this area in which the occlusion is only partial. However the final result is practically the same so long as the area contains regions of total as well as partial stenosis.

Our histologic study of the lung of the guinea-pig discloses the following prime causative factors in the production of acute serum-anaphylactic-shock, consequent upon an occlusion of the lumen of the secondary bronchi:

1. Tonic contraction of the smooth muscle.
2. Greatest relative (to diameter of lumen) amount of smooth muscle at this level.
3. Normally thicker mucosa and greater degree of folding of same relative to lumen.
4. Normal presence of small amount of mucus.
5. Extra-mucosal œdema and congestion (this more probably result of anaphylactic death than contributory cause).

The explanation of the recovery of a small per cent of sensitized guinea-pigs is probably to be found in individual anatomical or physiological variations and accidents of experimentation.

The recent work of Schultz¹ (11) has shown that serum-anaphylaxis is essentially a matter of hypersensitization of smooth muscle generally. When the second injection of horse serum is given to the guinea-pig, the smooth muscle everywhere appears to react similarly (contracts). But only at the level of the bronchial tree above indicated is the maximum amount of muscular contraction sufficient to produce complete occlusion of the lumen. The common effect, then, is apparently fatal only for guinea-pigs, due to a peculiar anatomical condition of the bronchial tree.

After a certain period, sensitized white mice react to a second injection in other respects similar to guinea-pigs (*i. e.*, increased peristalsis, contraction of the bladder, and increased irritability of the skin)—only the fatal respiratory symptoms are lacking. This result is clearly due to the fact that the mucosa of the bronchial tree of the mouse is nowhere sufficiently thick or folded,

¹ That smooth muscle is a very important factor in connection with the physiology of phenomena observed in animals treated with large doses of serum has been shown by several different methods. Soon after his first joint paper on anaphylaxis, Schultz demonstrated to several of his colleagues in the Hygienic Laboratory that the fall in blood pressure in animals injected with protein is attributable to the heart. These results with cats, dogs, and guinea-pigs have remained unpublished so as to duplicate and confirm the results by different methods. It, however, was proved that:

(1) The arterial system in response to both animal and vegetable protein constricts and at first a fleeting rise of blood pressure may be recorded. Secondary factors then enter causing a fall of blood pressure so marked as to mask all evidence of this vaso-constrictor action.

(2) This secondary factor is the heart itself which in response to the diminished flow through the constricted coronary and pulmonary arteries beats at first with less force and later at a slower rate.

(3) Cardiograms taken simultaneously with the blood-pressure show clearly that there is a relation between the fall in blood pressure and the ventricular output. And cardiograms of the excised heart show that with the diminished ventricular beat there is a diminished flow through the coronary vessels.

(4) The fall in blood pressure observed in all anaphylactic animals is attributable primarily to the heart, the lethal dose of the protein reacting with the receptive substance of (1) the smooth muscle and (2) possibly also with that of the cardiac muscle itself.—W. H. S.

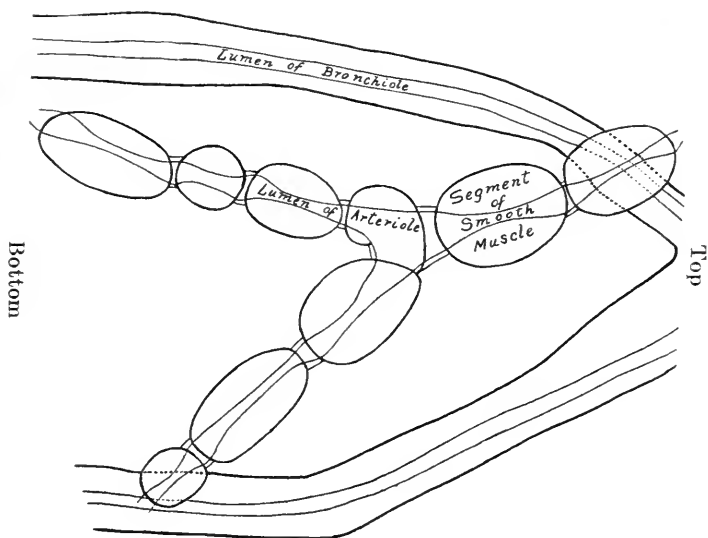


FIG. 5. Camera lucida outline sketch of bronchiole and arteriole at points of division. The musculature of arteriole is arranged in ellipsoidal and spherical segments, the intervals being spanned by a thin layer of circularly disposed smooth muscle fibers continuous with the inner layer of the segments. $\times 75$.

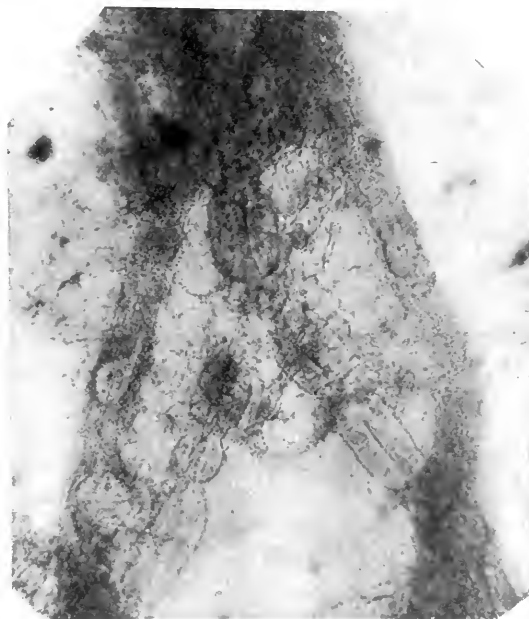


FIG. 6. Photomicrograph of preparation (unstained toto mount) illustrated in fig. 5.

relative to the amount of muscle and the diameter of the lumen, to produce occlusion under the amount of constriction produced by the contracting musculature.

Another peculiar anatomical feature—unique for the lung of the guinea-pig as far as we can learn—remains to be described. It is possible that this also may be a contributory factor in the production of the complex of anaphylactic phenomena. This

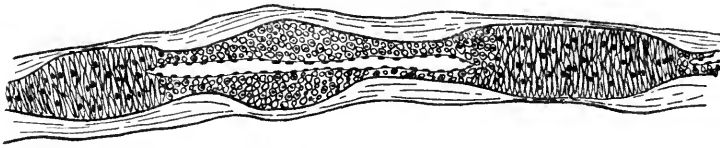


FIG. 7. Camera lucida drawing of oblique longitudinal section of arteriole, showing the segmental arrangement of the musculature of the media, the circular disposition of the individual fibers, and the comparative width of lumen and loose adventitia. $\times 100$.

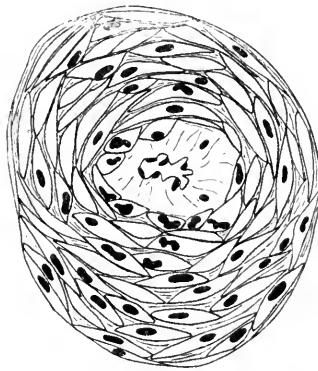


FIG. 8. Transverse section of arteriole of normal lung through muscle segment. $\times 400$.

peculiarity is especially pronounced in the case of precapillary arteries (arterioles) accompanying the smaller bronchial tubes and the bronchioles, though it is characteristic in a small degree of all arteries of the lung. Under low magnification (fig. 5) and in section (figs. 7 and 8) these "beads" are seen to be ellipsoidal masses of circularly disposed smooth muscle fibers. The short intervals between successive muscle "beads" is spanned by a thin layer

of circularly disposed smooth muscle, continuous with the inner layer of the "beads." The lumen of these arterioles is practically obliterated in both the normal and anaphylactic lung, and free of blood. The normally small calibre of the lumen is probably further reduced by reason of the muscular contraction, due in one case to the action of the fixing fluid, and in the other possibly to the second injection of horse serum. A more detailed description of the structural peculiarities of these arterioles, and a discussion of them as a possible factor in the production of the vascular condition of the anaphylactic lung, will be reserved for a later and more extensive paper.

Briefly summarizing our results, it may be said that:

1. The cause of sudden anaphylactic death in guinea-pigs is asphyxia, caused by occlusion of the secondary or tertiary bronchi at the level indicated in fig. 1. This occlusion is a result of the folding and dove-tailing of the mucous membrane brought about by tonic contraction of the smooth muscle of the larger bronchi.

2. The lumina of the tertiary and smaller bronchi may be reduced but this simply lessens the respiratory volume by increasing the resistance to the air. The walls of the smaller bronchi being relatively poor in mucous membrane, occlusion is less apt to occur.

3. The alveolæ, their ducts and the bronchioles, are not only open, but distended, in the anaphylactic lung so that the whole lung volume is so increased as to fill the chest cavity.

4. Œdema is present in anaphylactic lungs in the region of the bronchial tree.

5. Sensitized white mice show many anaphylactic symptoms observed in guinea-pigs; increased irritability of the skin, urination, and defecation, low temperature, low blood pressure, marked changes in respiration, lesions in the ears and skin, and even collapse and death; but death never occurs from asphyxia. The right heart of recently dead animals is gorged with blood as is that of guinea-pigs, but the lungs are collapsed as in asphyxiated normal mice.

6. The respiratory changes in many respects resemble those observed in guinea-pigs nearly dying from anaphylactic shock. The

forced breathing can readily be accounted for by the resistance offered by the partially constricted bronchi due to contraction of the smooth muscle in their walls. The lumina, however, remain open because there is not sufficient mucous membrane present, relative to calibre of tube and amount of muscle, to cause the folding observed in guinea-pigs.

7. Since the pulmonary veins are filled with blood and the pulmonary arteries empty (the right heart being full) the peculiar arrangement of the muscle bands in the arteries of guinea-pigs probably constrict and force the blood into the less resisting structures and perhaps play an important part in hindering the proper pulmonary circulation.

8. The bronchi of the lungs of mice, cats, and man (probably also of dogs and rabbits) are in comparison with the homologous structures of the guinea-pig's lung, relatively poor in mucous membrane. This probably accounts for the almost complete absence of death from asphyxia. The occasional sudden death from serum in man is perhaps due to abnormal development or condition of the mucous membrane and smooth muscle which latter is hyperirritable; the same applies to the lungs of asthmatics. The forced respiration observed in all of these animals, however, is due mostly to the decreased size of the lumina of the bronchi caused by the contraction of the smooth muscle in their walls in response to the stimulating action of the injected serum.

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SCIENTIFIC PROCEEDINGS OF THE AMERICAN
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The Pharmacology of Oil of Chenopodium. William Salant. From
the Pharmacological Laboratory, Bureau of Chemistry, United
States Department of Agriculture.¹

The experiments were carried out on cats, rabbits, guinea pigs,
mice and dogs.

Experiments on cats: The oil was given in aqueous emulsion
by mouth through a stomach tube. One to one and a half hours
after its administration symptoms of intoxication usually appeared.
The cats became irritable at first, then depressed. Later paraly-
sis supervened; in some cases convulsions alternating with par-
alysis were observed. Coma set in late in the course of intoxi-
cation. It usually appeared about a day after the oil was given.
When large doses, 0.6 cc. per kilo, are fed death occurs within 18
hours. After smaller doses the outcome may be delayed con-
siderably, sometimes fully 48 hours elapsed between the adminis-
tration of the drug and the death of the animal. The minimum
fatal dose in our experiments was about 0.2 cc. per kilo. After
the administration of 0.1 cc. of oil of chenopodium per kilo by
mouth, cats survived without showing any symptoms.

Rabbits and guinea pigs are much more resistant to this sub-
stance than cats. Six-tenths of a cubic centimeter of the oil given
by mouth failed to induce any symptoms. In well fed rabbits

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0.9 cc. per kilo proved fatal within three hours. When administered subcutaneously the toxicity was about twice as great: the fatal dose was about 0.4–0.44 cc. per kilo. In one instance 0.3 cc. proved fatal. In this case the symptoms developed about 20 hours after the chenopodium was administered. Convulsions were observed at first, followed by forced movements and complete paralysis and coma.

Experiments on guinea pigs indicate that these animals vary considerably in their resistance to chenopodium. The subcutaneous injection of 0.27 cc. per kilo proved fatal to some individuals, others survived doses which were larger by 50 per cent. The symptoms are the same as in rabbits.

The active principle, ascaridol, $C_{10}H_{16}O_2$, isolated from the oil by Mr. N. G. Nelson of the Bureau of Chemistry, showed that this substance was about twice as toxic as the oil. Experiments on rabbits have shown that 0.18–0.20 cc. may be fatal, although some rabbits survived such doses. The test made to determine whether tolerance could be established in rabbits proved negative. I found on the contrary, that non-toxic doses when repeated in a day or two, were fatal, thus pointing to a cumulative action. In some rabbits the administration by mouth of 0.3 cc. per kilo daily for two days caused death. Other animals tolerated a larger number of injections. Chenopodium is a powerful circulatory depressant: 0.01 cc. per kilo caused a fall of blood pressure in a dog, of 35 to 40 per cent, which remained stationary for several minutes. A second injection, ten minutes after the first caused a fall of blood pressure which amounted to only about 12 per cent.

Experiments were also made to determine whether toxicity is modified in starvation and in chronic alcoholism. Cats which were starved 6–9 days seemed to be more susceptible than well fed animals. The same was true of guinea pigs. In chronic alcoholism, on the contrary, the resistance was decidedly increased. It is interesting to observe in this connection that when ascaridol, or the oil, was reduced with ferrous sulphate its toxicity was much diminished. This was shown by a study of its effect on general toxicity in cats and rabbits, as well as by experiments on the effect on blood pressure.

Physiological Assay of Ergot. C. W. Edmunds and Worth Hale.
From the Pharmacological Laboratories of the United States
Public Health and Marine Hospital Service and of the Uni-
versity of Michigan.

An examination of two series of fluid extracts of ergot consisting of six preparations by chemical and physiological methods of assay showed a very close agreement between results obtained on the cock's comb with those on the uterus. The blood pressure estimations agreed fairly well in certain cases, but there were some glaring discrepancies which would seem to contraindicate the adoption of this method of assay. The estimation of the cornutin by Keller's method also showed some agreement with the results obtained by physiological methods, but here, too, discrepancies occurred. The weight of the substances soluble in benzol offered no indication of the strength of the preparation.

Further Data Relating to the Value of Phenolsulphonephthalein in Estimating the Functional Capacity of the Kidney. L. G. Rowntree and J. G. Geraghty. From the Pharmacological Laboratory, Johns Hopkins University and the Genito-urinary Clinic of the Johns Hopkins Hospital.

Data have been collected now in about fifty cases of unilateral disease of the kidneys. In no instance has the test been at fault, opportunity for confirming the findings of the test being afforded in nearly every instance, by a subsequent operation. Instances were reported where the diagnosis, which later proved correct, was made on the strength of the test and where without the test the true condition of the kidney would not have been suspected. In all operated cases the patients have had uneventful recoveries, the function of the removed kidney being gradually taken on by the remaining kidney.

It was shown that in healthy dogs the removal of one kidney is followed by a normal or only slightly lower function for a period of one-half to one hour, but that later the function, as indicated by the test, begins to decrease. In the clinical cases it was

shown that the function of the remaining normal kidney is about the same as it was prior to operation, if studied three days after operation. At the fifteenth day it has somewhat increased and in three weeks is usually as great as that of the two kidneys prior to operation.

As a result of the study of a large number of cardiac, cardio-renal and renal cases, it has been found that it is possible to differentiate types of cases in which the renal disease is advanced and is the chief factor in the production of the clinical picture from the cardio-vasculo-renal cases in which the renal condition is a secondary and minor factor.

The results obtained in some cases of acute nephritis, in chronic parenchymatous and in chronic interstitial nephritis were reported, and were seen to be in keeping with the results already published.

Several more cases of uraemia in which there was no secretion of the drug at all or in which it was reduced to a minimum have been observed. Death followed in all these cases and autopsies revealed extreme grades of nephritis of different varieties.

The influence of various diuretics on the kidney function as indicated by the tests was considered and also the influence of blood pressure, water and salt excretion on the excretion of the sulphonaphthalein.

The results of further studies with the test have only succeeded in further demonstrating its great value in diagnosis in clinical medicine and surgery and in increasing the reliance to be placed upon its findings.

The Vaso-motor Supply of the Lungs. Horatio C. Wood, Jr.
From the Pharmacological Laboratory, Medico-Chirurgical
College, Philadelphia.

Doctor Karsner, at the suggestion of the author, carried out a series of histological researches in which he succeeded in demonstrating the presence of nerve fibrillae in the muscular coat of the pulmonary artery. This is very strong evidence in favor of the belief that the pulmonary circulation is under vaso-motor control.

The theory was suggested that the vaso-motor centers are located

in the thoracic portion of the spinal cord. This would explain the slowness of the rise of pressure in the pulmonary artery under asphyxia. In order to test this theory, the medulla was destroyed by injecting paraffine into the cranial end of the carotid artery. After this, asphyxiation produced a rise in the pulmonary blood vessels with a fall in the carotid.

The effect of a number of drugs on the pulmonary pressure was tested with the following results:

Adrenalin produced a rise in the pulmonary pressure which begins simultaneously with the rise in the carotid pressure, and cannot, therefore, be due solely to damming back of the blood.

Ergot produced a rise in both the carotid and the pulmonary arteries, but the pulmonary elevation persists even after the injection of sufficiently large doses to cause a secondary fall in the carotid pressure.

Ether produced in some cases a moderate, and in others a very marked increase in the pressure in the pulmonary artery, with, sometimes, a fall, and sometimes a slight elevation in the carotid.

Alcohol caused a slight increase in the pulmonary pressure.

Quebrachine, the dominant alkaloid of *Aspidosperma*, produced a moderate increase in the pulmonary pressure.

The nitrites caused a moderate increase in the pressure of the blood vessels of the lungs accompanying the marked fall in the systemic pressure.

The Modifying Influence of Anemia on the Actions of Some Well-known Drugs. Carl J. Wiggers. From the Physiological Laboratory, University of Michigan.

There are on record a number of researches showing that anatomical removal, pathological destruction or chemical alteration of structures may prevent or precisely reverse the well-known actions of drugs; but that functional disturbances such as may be induced by anemia may act similarly has not been brought out.

Doses of *adrenalin* that slow the heart in normal animals lose this function in anemia due to a depression of the cardio-inhibitory center. This causes a greater actual as well as percentile rise in arterial pressure.

Digitalis preparations retain their strengthening action but lose their slowing effect on the heart, following the loss of blood.

Nitrites and *nitroglycerine* in normal animals cause a simple pharmacological action, viz., they dilate the blood vessels and consequently cause a fall in arterial pressure. In larger doses they decrease the amplitude of cardiac contraction. The fall of pressure sets into action two physiological compensatory mechanisms, viz., an acceleration of the heart and an increase in amplitude. During hemorrhage no acceleration takes place and the heart amplitude, if changed, is depressed, as in perfusion experiments.

This induces two important modifications in the circulation: the arterial pressure continues low for a longer time unless compensated by increased respirations and the typical increase of pressure in the pulmonary vessels observed in normal animals is changed to a fall, explaining perhaps the records of favorable clinical results during hemoptysis.

Ergotoxin loses its pressure raising action during hemorrhage.

Morphine so free from circulatory action when given in therapeutic doses to normal animals causes, when given to animals whose respirations are dyspneic from the anemia of hemorrhage, a slowing of breathing and a consequent fall in arterial pressure which is not infrequently fatal.

Chloroform apparently causes less depression of the heart after a loss of blood than in normal animals for the reduction of the amplitude of ventricular contractions occasioned by a certain dose is less after bleeding than before and the dose that may be just survived is greater.

Further Data Relating to the Use of Antimony-Thioglycollic Acid Compounds in the Treatment of Experimental Trypanosomiasis. L. G. Rowntree and J. J. Abel. From the Pharmacological Laboratory of Johns Hopkins University.

In a recent publication the results obtained by the authors up till July 1, 1910, in the treatment of experimental trypanosomiasis by means of sodium antimony-thioglycolate and the triamide of antimony thioglycollic acid were given. These former results

were briefly presented and discussed and then the subsequent history of these animals presented.

Of 158 infected rats treated 55 were living at the time of publication, July 1, 1910. These were left with the laboratory attendant during the summer months, and on October 1, 1910, 15 were still living. In many instances the rats were recognized to be sick prior to death, but their blood was found to be free from trypanosomes. Six rats have died since October 1st, but in no instance was there a relapse—the animals dying of pneumonia or some disease other than trypanosomiasis.

Of the total number treated

37 lived more than 100 days
 21 lived more than 150 days
 12 lived more than 200 days
 9 lived more than 250 days
 6 lived more than 300 days
 3 lived more than 350 days
 1 lived more than 1 year.

At the time of this report 9 are still living; the particulars concerning which are included in the following table:

DISEASE	DATE OF INFECTION	HOURS ELAPSING BEFORE TREATMENT	NUMBER OF INJECTIONS	DOSE IN MGS.	DRUG GIVEN	LIVING AFTER
		<i>Hours</i>				<i>Days</i>
Surra of Mauritius	1910 March 28	48	12	2	{ Sod. ant. thioglycollate }	278
Surra of India	May 1	72	3	6	{ Triamide of ant. thioglyc. acid }	214
Surra of Mauritius	May 31	96	3	5	{ Sod. ant. thioglycollate }	127
Dourine.....	March 4	96	12	5	Triamide	305
Surra of India	Feb. 9	48	14	3	{ Sod. ant. thioglycollate }	328
Nagana.....	Feb. 19	120	18	5	Triamide	318
Nagana.....	Jan. 4 1910	24	4	1	{ Sod. ant. thioglycollate }	361
Nagana.....	Dec. 21 1909	48	3	2	{ Sod. ant. thioglycollate }	375
Nagana.....	Jan. 4 1910	24	1	3	{ Sod. ant. thioglycollate }	362

Of 7 rabbits treated only one is still living after 286 days, this animal receiving treatment on the fourteenth day following its infection. Of the others one lived 203 and another 236 days.

A dog given a prophylactic dose at the time of its infection was killed seven months later, the disease not having developed.

A dog infected on November 19, 1909, was treated with an arsenic preparation, dimethylamidoarsenoxide, two weeks later, at which time its blood contained numerous trypanosomes and the animal was blind as the result of a keratitis, set up by the disease. The trypanosomes disappeared from the blood and the keratitis quickly cleared up. The dog has remained in a perfectly normal condition and repeated subinoculations have proved negative. The dog is in perfect physical condition now after the lapse of $13\frac{1}{2}$ months. The arsenic preparation referred to, however, has proved too toxic for general use.

These and other antimonic and arsenic compounds have been tried in the treatment of experimental relapsing fever of mice (*Recurrans* of Koch) and in chicken spirilloses, but without the desired effect. The specificity of their action against trypanosomes is striking.

The Action of G-Strophanthin on the Isolated Mammalian Heart.

Chas. W. Greene. From the Physiological and Pharmacological Laboratory, University of Missouri.

The action of g-strophanthin was determined on the rabbit and on the cat heart isolated by the Bock method of establishing a cross circulation between the carotid artery and the jugular vein, all other arteries being ligated and artificial respiration being maintained. The heart so isolated is very susceptible to the action of the strophanthin. Very small doses must be used to avoid immediate toxicity. Doses of 0.01 mgrm., 0.01 to 0.02 per cent in physiological saline solution, were slowly injected into the blood stream. The strophanthin caused little change in the heart rate, usually a slight slowing. On the other hand there was a rise in arterial pressure. The rise in pressure was relatively slight in rabbits but stronger in cats. Bock's method is primarily

one to test the volume of the heart discharge per unit of time, hence these results show that g-strophanthin when its action is restricted to the local cardiac mechanism increases the volume of the heart beat.

G-strophanthin is remarkably toxic to such isolated hearts. A total dosage of from 0.03 to 0.06 mgrm. is sufficient to bring on permanent incoördination of contractions from which the hearts do not recover.

The Action of Acetanilide on Isolated Cardiac Muscle. Gloria Carr.
From the Physiological and Pharmacological Laboratory,
University of Missouri.

Cushny states that the antipyretics accelerate the heart of the frog and of the mammal, then cause slowing and irregularity, and that these alterations are, "entirely independent of the inhibitory mechanism and are due to the direct effect of the drug on the cardiac muscle." My experiments attempt to determine the

Table showing the action of acetanilide on strips of ventricular muscle of the terrapin. Acetanilide dissolved in Ringer's solution. The effect is expressed as percentage change from the normal taken just before the application of the drug.

EXPERIMENT NUMBER	PER CENT OF THE SOLUTION	TIDAL	CHANGE AFTER 1 MIN. IN PER CENT OF NORMAL		CHANGE AFTER 1.5 MIN. IN PER CENT OF NORMAL		
			Rate	Amp.	Rate	Amp.	
34	0.1	1st	100	100	105	100	{ Very slight periodic irregularity in amplitude Very slight periodic irregularity in amp. and rate.
		2nd	100	103	107	98	
31	0.12	1st	125	101	104	105	{ Slight irregularities in rate. Slight irregularities in rate.
		2nd	112	103	87	103	
24a	0.25	2nd	55.8	109	50	114	{ Cessation of contractions, 1.8 min. Cessation of contractions, 1.5 min.
		3rd	74	107	66	114	
25	0.4	1st	133	100	111	102	{ Cessation of contractions, 1.2 min. Cessation of contractions, 2 min.
		2nd	120	101	—	—	
24b	0.5	1st	83	122	83	128	{ Great slowing—no complete cessation of contractions.
		2nd	138	103	111	101	

influence of acetanilide on isolated cardiac muscle. Apex strips of the ventricle of the terrapin, and sinus strips including the left vena cava were used. The technique given in Greene's Experimental Pharmacology was followed. Weaker Ringer's solution (0.7 per cent NaCl + 0.009 CaCl₂ + 0.01 KCl) for the normal solution and as a vehicle for the drug. Heart strips were subjected to acetanilide in the following percentages: 0.1, 0.12, 0.25, 0.4, and 0.5.

The tables show the trend of the varying results obtained upon the applications of solutions of the drug. The weaker solutions were slow to act and rather inconstant in their effects. The rate especially varied greatly, but after a second trial the greater number of tests resulted in a slowing. With the intermediate strengths the decrease in rate came on more promptly and in some cases it ceased entirely. The higher percentages were manifestly toxic as judged by the stopping of the rate.

Strips from the sinus, mounted with the ventricular strips, gave good contractions in the normal Ringer solution, both as to the fundamental rhythm and the tone waves. These contractions were both sharply diminished or completely stopped by acetanilide solutions of all the strengths used.

The Elimination of Creatin and Creatinin after the Administration of Caffeine. William Salant and J. B. Rieger. From the Pharmacological Laboratory, Bureau of Chemistry, United States Department of Agriculture.¹

Experiments on rabbits indicate that the urinary creatin is increased after the administration of caffeine. One decigram per kilo injected subcutaneously into well fed rabbits which received oats or carrots was followed by a rise in the output of creatin, amounting in some cases to 100 per cent or more as compared with that obtained in the control periods. In a number of rabbits caffeine in increasing doses was given by mouth. Fifty milligrams fed daily for several days failed to produce any notice-

¹Presented by permission of the Secretary of Agriculture.

able effect in the creatin output. Larger doses, 100 to 150 milligrams per kilo daily caused an increased elimination of creatin, especially when the doses were repeated. The rise in the creatin output often persisted for several days after caffeine was discontinued and sometimes appeared a day or two after its administration was begun. Neither the increased diuresis nor the diminished appetite could be regarded as a factor in accounting for the greater output of creatin. The elimination of the creatinin was variable in some rabbits, but in most of them it was practically not affected by caffeine.

The Influence of Caffeine on Protein Metabolism in Dogs, with some remarks on Demethylation in the Body. William Salant and I. K. Phelps. Bureau of Chemistry, United States Department of Agriculture.¹

The experiments were carried out on two healthy dogs which received a mixed diet containing protein corresponding to 0.75 grams nitrogen per kilo. The protein was given at first in the form of plasmon, but meat was substituted later in the experiment. Fifty to seventy-five milligrams of caffeine per kilo were given daily by mouth in capsules, for eight and four days respectively. The results obtained in one dog indicate that nitrogen retention observed in the fore period was not disturbed by caffeine feeding. The other dog which was in nitrogen equilibrium showed a distinct loss of nitrogen, indicating increase of protein catabolism associated with caffeine feeding. In both dogs the elimination of total nitrogen was markedly increased during the after period when caffeine was withheld. On resuming the administration of caffeine, when 75 to 100 milligrams per kilo were given, nitrogen retention was observed in the first period of four days in both dogs, followed by nitrogen equilibrium in the next period. Analysis of sulphates showed a decided increase of the ethereal sulphates when the administration of caffeine was suspended. The amount of purin nitrogen per kilo obtained in four days on

¹Presented by permission of the Secretary of Agriculture.

one dog after the ingestion of 200 milligrams of caffeine per kilo during this period showed an increase of three to five milligrams per kilo. In the other dog the amount of purin nitrogen was 16 milligrams per kilo greater after the administration of the same amount of caffeine per kilo during an equal interval of time. When the administration of caffeine was resumed after an interval of eight days an increased elimination of purins was observed, 200 per cent in one case and 60 per cent in the other, although the amount of caffeine given was only 20 per cent greater. The resistance to caffeine was found to vary with the amounts of the urinary purins eliminated. This was corroborated by us in experiments on rabbits.

On Intramuscular Absorption. J. Auer and S. J. Meltzer. From the Department of Physiology and Pharmacology, Rockefeller Institute.

In a previous communication the authors, by using adrenalin and other substances, furnished experimental evidence that the effect of an intramuscular injection is near that of an intravenous and is far superior to that of a subcutaneous injection. Patta denied this fact and Wallace who corroborated the fact explained it by the assumption that the absorption takes place through torn veins. In repeating the former experiments we have, among other things, tied a wide glass cannula in the lumbar muscles of rabbits, established by test the absence of blood at the bottom of the cannula in the muscle, left the cannula open for forty minutes, then filled it up with adrenalin, clamped the rubber tubing of the cannula and injected through the rubber into the cannula one cubic centimeter of adrenalin. Very soon a high rise of blood-pressure set in which lasted a long time, in one case longer than half an hour; that is, the increase of blood-pressure lasted by this method longer than it usually lasts by the intravenous method.

In this series of experiments it was established at the same time that an injection into the gluteal muscle gave either no rise at all or only a small rise; at any rate it gave no constant results.

Constantly favorable results can be obtained apparently only when the injection is given in massive muscles surrounded by a strong fascia.

Experiments with Salts of Aluminium and Beryllium. William J. Gies. From the Department of Physiological Chemistry, Columbia University.

In continuation of our work on the toxicity of aluminium,¹ Prof. Steel has found, in experiments on dogs, that aluminium is absorbed from aluminized food into the blood; that such aluminium does not accumulate in the blood; and that intravenous injection of small doses of aluminium chloride is followed by the elimination of aluminium in the feces. Aluminium-protein compounds have been prepared by Mr. M. G. Herzfeld and will shortly be made the subjects of pharmacological study.

Dr. W. H. Welker and Miss Alice Knox have found that white lupin seedlings exhibit slight *initial development* of the roots in solutions of beryllium sulphate containing an $\frac{M}{256}$ proportion of that substance. There was *no growth* after 24 hours in $\frac{M}{8192}$ solutions. *Stimulation* of growth occurred in concentrations ranging from $\frac{M}{131,172}$ to $\frac{M}{524,588}$. Miss Seaman has found that sucrase from yeast is very active in the presence of large proportions of beryllium sulphate (*e.g.*, 2 per cent). Her studies of the influence of beryllium sulphate in dogs, which are now in progress, indicate, in a preliminary way, that beryllium is somewhat more toxic than aluminium when introduced per os and that, in moderate doses, it slightly increases nitrogenous catabolism.

We are greatly indebted to Dr. Charles L. Parsons for the beryllium sulphate we are employing in this work.

Further Observations on the Action of Iodoso- and Iodoxybenzoic Acids. A. S. Loevenhart. From the Pharmacological Laboratory, University of Wisconsin.

¹House and Gies: Proceedings of the American Physiological Society, American Journal of Physiology, 1906, 15, p. XIX.

The Control of Strychnine Poisoning by Means of Insufflation and Ether. T. S. Githens and S. J. Meltzer. From the Department of Physiology and Pharmacology, Rockefeller Institute. (Is published in full in the present number of this JOURNAL).

The Rôle of the Portal Circulation of the Liver in Bile Formation and Jaundice. Carl Voegtlin and Bertram M. Bernheim. From the Department of Pharmacology and the Hunterian Laboratory of the Johns Hopkins University.

In dogs with an Eck fistula (anastomosis of the portal vein with the vena cava inferior and ligation of portal vein near hilus of liver) ligation of the common bile-duct is not followed by jaundice. The bile pigments which the urine contains soon after the operation disappear within a few days. The animals live in apparently good health, whereas simple obstructive jaundice always leads to death within a month. The blood of such dogs contains the normal amount of hemoglobin and red cells which is in striking contrast with the findings in jaundiced dogs. The liver does not show the necrotic changes found in obstructive jaundice. All these facts can be explained by assuming that *in case the portal blood does not pass through the liver*, less bile is formed. On the other hand these experiments tend to demonstrate that the secretion of bile into the duodenum is not essential for the maintenance of life, nor for the removal of metabolic end products.

Tetanic Convulsions in Frogs Produced by Acid Fuchsin and Their Relation to the Problems of Inhibition in the Central Nervous System. H. G. Barbour and J. J. Abel. From the Pharmacological Laboratory, Johns Hopkins University. (Has appeared in No. 3. vol. II of this JOURNAL).

The Action of Sodium Chloride upon the Phenomena Following the Removal of the Parathyroids in Dogs. D. R. Joseph and S. J. Meltzer. From the Department of Physiology and Pharmacology, Rockefeller Institute. (Is published in full in the present number of this JOURNAL).

AN EXPERIMENTAL STUDY OF CAMPHORIC ACID

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The introduction of camphoric acid by Fürbringer (1) as a means of preventing phthisical sweating led many clinicians to investigate its properties. Previous to this it was used by Fürbringer and others as an antiseptic against tubercle bacilli. While engaged in a clinical study of its antiseptic value, Wittkowski, working under the direction of Fürbringer, discovered its efficacy in stopping the night sweats of tuberculosis and further study by Fürbringer led him to believe that camphoric acid was as efficient as atropine in controlling this symptom of the disease.

Other early workers who have investigated its properties are Bohland (2), Leu (3), Niesel (4), Dreesman (5) and Hartleib (6). Stockman (7) used it in non-tubercular as well as tubercular cases with equally good results and he concluded that it acted as efficiently as atropine in phthisical cases while in obstinate cases it was inferior to picrotoxin. Wood (8) also found that in many cases of tubercular sweating only a few doses were required to give immediate and in some cases lasting relief. More recently Tyrode (9) reported the results of clinical observations made by several of his colleagues who concluded that it has no value in checking the night sweats of phthisis. In some cases they found that the patients became worse under the camphoric acid treatment.

From the pharmacological standpoint there is a wide difference of opinion as to its mode of action, also as to its value in checking sweating produced experimentally. Stockman (7) believed that it checks the sweating by paralyzing the nerve

ends in the same way as atropine. Kobert (10) advanced the theory that the sweating in pulmonary diseases is due to deficient respiration. Hence any drug which stimulates the respiratory center would cause a disappearance of this symptom. He ascribed a camphor-like action to camphoric acid, which resembling picrotoxin would combat the sweats by stimulation of the medulla. Schmiedeberg (11) gives it a place among the nerve stimulants.

The recent work of Fugitani (12) and Tyrode (9) on the pharmacology of camphoric acid being so widely divergent the necessity of further investigation along experimental lines is made evident. The first experimental work was done by Wagener (13) who found in two experiments on cats that camphoric acid injected as the sodium salt has an action similar to camphor, namely to produce periodic convulsions of an epileptiform nature and a rise in blood pressure, while in frogs a curara-like action is produced. Dreesman (5) tried to check the sweating in cats produced by 0.010 gram of pilocarpine with from 1 to 3 grams of camphoric acid, but was unsuccessful. Hayashi (14) gave camphoric acid neutralized with sodium carbonate to rabbits per stomach and obtained a slight fall in temperature. If given in large doses as an acid the temperature would drop several degrees in the course of a few hours. However, the death of the animal followed later. Stockman (7) repeated the work of Dreesman but obtained opposite results. He succeeded in stopping the sweating produced in cats by one-eighth grain of pilocarpine nitrate with 15 grains of sodium camphorate, and from this he concluded that camphoric acid paralyzed the terminations of the secretory nerves in the sweat glands. Using 2 to 4 grain doses on frogs, only slight depression was obtained and occasionally an increase of reflexes, while 75 grain doses in rabbits gave practically the same symptoms. Fugitani (12) found that in the frog with sodium camphorate in doses up to 0.1 gram nothing abnormal was obtained except local irritation. In doses of from 0.15 gram to 0.2 gram there developed in the course of an hour a gradual depression accompanied by a decrease in respiration. He obtained neither convulsions nor increased re-

flexes. Larger doses of 0.4 gram or more produced a prompt central paralysis, the motor end plates not being affected. The heart rate was diminished and the force lessened. He further found that it increased the blood pressure in mammals and caused a marked increase in the depth and frequency of the respiration. No symptoms were produced in rabbits, cats or dogs even with 2 to 7 gram doses, except that in rabbits an increase in the respiration was noticed after an intravenous injection of 0.3 gram or more and that with doses of 1.5 grams a period of dyspnea occurred. The drug proved comparatively non-toxic in his hands. As it failed to check the sweating produced in cats by stimulation of the sciatics he concluded that it did not paralyze the peripheral nerves to the sweat glands.

Tyrode (9) using the sodium salt of camphoric acid found that it had no well marked pharmacologic action. In frogs 0.5 gram was required to give symptoms. These consisted of lessened movements followed by a gradual central paralysis, the heart being stopped in diastole. In a series of experiments on frogs with the heart exposed he obtained no specific effect even when solutions as strong as 20 per cent were used. The general effects were studied in rabbits and cats and no well defined symptoms were ever noticed even after 5 grams of sodium camphorate. Upon the respiration and blood pressure it was equally inactive. He obtained an increase in urinary secretion but attributed it to salt action. In determining its effects upon the nutrition in rabbits no marked changes were noted. The weight remained constant when it was administered to grown rabbits and increased normally in young animals.

In this investigation three specimens of camphoric acid were used. One was obtained from Schuchardt with a melting-point of 182°C . A second preparation obtained from Merck and Company had a melting-point of 187°C . The other sample obtained from local sources had a melting point of 182.5°C . All of these samples conformed to the requirements of the U.S.P. VIII, as to color, odor, solubility, reaction of aqueous solution to litmus, and absence of nitric acid.

Sodium camphorate (or the sodium salt of camphoric acid) was used entirely for the animal experiments, being made by neutralizing the acid with sodium carbonate or sodium hydroxide. As a further check upon the work sodium camphorate made by Merck and Company was used to confirm the earlier results.

Every preparation of sodium camphorate was tested as to its reaction toward litmus and its behavior to zinc and nickel salts, and all specimens met the requirements as stated by Fehling. *Neues Handwörterbuch der Chemie* 1875, II, 382.

ACTION UPON THE FROG

In the intact animal sodium camphorate injected into the ventral lymph sac in doses up to 0.010 gram per gram of body weight does not produce prominent symptoms other than a noticeable increase in respiration. If doses of 0.010 gram and up per gram of body weight were given, the animal immediately showed violent movements, which were no doubt due to the irritation produced by the solution. This was soon followed by irregular respiration and general depression. In from 5 to 40 minutes muscular twitchings would begin appearing first in the toes of the hind limbs and then becoming generalized. In many of the animals the reflexes were distinctly increased and convulsions which were usually of a tonic type occurred later, followed by paralysis and death in from one to several hours. In case smaller doses had been given the animal would live from eight to ten days.

The following protocol may be taken as typical of the result usually obtained.

11-29-09. Frog, Weight, 24 grams

- 1:40 Injected 1.8 cc. of a 20 per cent solution of Merck's sodium camphorate (0.015 gram per 1 gram body weight) into the ventral lymph sac.
- 1:41 Jumps about violently.
- 1:45 Respiration somewhat irregular.
- 1:50 Head bent, animal rather quiet.

- 1:58 Raises hind legs over body. Toes twitch violently, also violent muscular twitchings in hind extremities.
- 2:00 Twitchings over entire body, but especially in the extremities.
- 2:01 Reflexes not increased, violent twitchings throughout.
- 2:02 Tries to move, breathing slow and irregular.
- 2:10 Still twitches. Slight increase in reflexes.
- 2:15 Moves about with difficulty. Head down. Gasps.
- 2:20 Reflexes diminished. Twitchings continue but are less frequent and violent.
- 2:24 Breathing better. Sitting up in more natural position.
- 2:40 Still twitches. Breathing better. Moves about.
- 3:10 Twitchings less violent.
- 3:40 Respiration irregular. No increase in reflexes.
- 4:30 Sitting up. Respiration normal.
- 5:30 Same.

On the next day the frog was alive but drowsy, and on the third day death occurred.

Whenever paralysis appeared it was always found to be of central origin. The twitchings were not due to an action on the muscles as they never appeared in curarized animals.

In one series of frogs the brain and cord were pithed before the injection of the drug and in these animals no twitchings appeared. On the other hand if the pithing was postponed until after twitchings were present they immediately disappeared when the central nervous system was destroyed. The twitchings then were mainly of central origin but in a few animals in which the sciatics had been cut, very slight movements were noticed in the toes, indicating that there was in addition to the central action, also a very feeble peripheral effect. The entire central nervous system seemed to be stimulated in the frog although in many cases the twitchings would almost entirely disappear after the higher parts of the nervous system were destroyed.

The action on the frog's heart was studied in the intact animal and upon the isolated heart.

Intact animal. The animal was pithed and the heart exposed. In general any dose below 0.010 gram per 1 gram of body weight had little or no effect but with doses as large as 0.024 gram per 1 gram body weight a slowing of from ten to thirty beats was obtained in the course of

an hour. The slowing was not influenced by previous application of atropine.

Isolated heart. The excised organ was perfused with modified Ringer's solution made after the following formula:

NaCl 0.6 per cent; CaCl_2 0.02 per cent; KCl 0.0125 per cent.

The arterial cannula was held in approximately a perpendicular position so as to give the heart from 20-30 mm. of fluid to work against. The pressure of the perfusion fluid was kept constant at the height of about 70 mm.

One experiment will suffice to show the effect of both weak and strong solutions of the drug. (See Table on opposite page.)

The experiment shows us clearly that the drug has little effect upon the excised heart except when used in strong solutions and then causes a rapid and marked decrease in rate and a prompt decline in the efficiency of the organ. We may therefore reach the conclusion that sodium camphorate in moderate doses has little effect upon the heart of the frog.

Action upon Mammals. The effect of the drug upon higher animals was studied upon cats and rabbits.

ACTION ON THE CAT

1-27-10. Cat, Weight, 1.35 Kg.

- 1:55 Injected subcutaneously 8.7 cc. of a 20 per cent solution of sodium camphorate (1.29 G. per Kg.)
 - 1:58 Meows.
 - 2:04 Quiet.
 - 2:10 At ease. Respiration 35 per minute.
 - 2:30 No change.
 - 2:40 Respiration 34 per minute.
 - 3:00 Sitting quietly as usual.
 - 3:20 Respiration 38; more attentive to surroundings.
 - 3:40 Respiration 38; slight movement of ears. Pupils widely dilated.
 - 3:50 Respiration 35; not as attentive to surroundings as before.
 - 4:30 Respiration 35. Pupils still dilated. No increase in reflexes.
 - 5:00 No change.
 - 5:20 Pupils dilated. Animal quiet.
- 1-29-10, 10 A.M. Animal apparently normal. Pupils of normal size.

Experiment XXXV. November 10, 1909. Perfusion of Frog's heart. Heart atropinized before starting experiment

TIME	RATE OF HEART	FLUID PUMPED	REMARKS
	<i>per min.</i>	<i>per 5 min.</i>	
10:00	51	cc.	
10:05	45	48	
10:10	43	48	
10:15	41	45	
10:20	41	48	
10:22			$\frac{1}{50}$ per cent sodium camphorate in
10:23	42		Ringer's solution.
10:25	42	46	
10:30	42	47	
10:35	42	49	
10:36			Ringer's solution.
10:40	41	50	
10:45	41	46	
10:50	41	47	
10:52			$\frac{1}{25}$ per cent sodium camphorate in
10:54	41		Ringer's solution.
10:55	41	49	
11:00	40	37	
11:05	40	42	
11:10	39	37	
11:11			Ringer's solution.
11:15	39.	38	
11:20	40	39	
11:25	40	40	
11:26			$\frac{1}{2}$ per cent sodium camphorate in
11:28	38		Ringer's solution.
11:30	35	16	
11:33	27		
11:35	25	4	
11:37			Ringer's solution.
11:40	18	2	
11:45	19	2	
11:50	21	4	
11:55	30	10	Discontinued experiment.

ACTION UPON THE RABBIT

The above experiment was duplicated upon the rabbit giving a large dose of sodium camphorate subcutaneously and the results were in every way similar to those obtained in the cat. Subcutaneous injections of sodium camphorate in the cat and the rabbit may then be said to have very little effect except to produce a slight increase in rate of respiration and dilation of the pupil. It is possible that the absence of symptoms in these animals might have been due to a very slow absorption so in order to avoid this factor I isolated an ear vein in the rabbit and inserted a cannula, thus injecting the drug directly into the circulation.

3-30-10. Rabbit, Weight 2.07 Kg.

- 2:13 Injected intravenously 25 cc. of a 20 per cent solution of sodium camphorate (2.4 grams per Kg.)
- 2:17 Animal quiet. Respiration 46. Pupils equal, 9 mm. in diameter.
- 2:27 Eating. Respiration 52.
- 2:30 Urinates. Moves about cage. Drinks water.
- 2:40 Quiet. Respiration 42, but deeper.
- 3:00 Respiration 42, condition not changed.
- 3:15 Respiration about same. Urinates.
- 3:30 Respiration 50; deep and full.
- 3:45 Respiration 52; drinks water.
- 3:50 Respiration 70, very deep. Quiet in corner of cage.
- 3:58 Clonic convulsions. Pupils 5 mm. in diameter.
- 3:59 Respiration 100, not so deep. Animal depressed. No increase in reflexes.
- 4:03 Respiration 92, labored.
- 4:30 Pupils not so much contracted. Respiration 74. Animal not so depressed as before.
- 4:45 Respiration 80. Animal sitting up. Pupils still somewhat contracted. On the three following days the animal was apparently normal, but on the fourth day after the injection it was found dead in its cage.

To a second rabbit a slightly larger dose (2.7 grams per Kg.) was given which gave similar results with the exception that

death occurred during the convulsion which came on two and a half hours after the drug was injected.

The drug is thus shown to be relatively non-toxic when given subcutaneously, and to possess well marked physiological properties when it is injected into the blood stream.

Perhaps the most striking feature of the poisoning is the late appearance of the convulsions even when the drug is injected directly into the circulation. This would seem to indicate clearly that they are not produced by camphoric acid itself but by some other substance which is slowly formed from it in the body.

ACTION ON THE SALIVARY GLANDS

Wagener (13) obtained a flow of saliva in cats and to ascertain whether it had any effect upon the salivary secretion I carried out one experiment upon the dog, anaesthetizing the animal with morphine and chloretone. After dissecting out the submaxillary duct a cannula was inserted into it and in this way the flow of saliva could be observed. The drug was injected into the femoral vein. To guard against a possible error in technic the chorda tympani was also isolated and stimulated to show that the lumen of the duct was patent. The results were entirely negative even with large doses of sodium camphorate (total of 5 grams). Chorda stimulation always produced a flow of saliva while injections of the drug were entirely inactive.

DIURETIC ACTION

In the above experiments it was observed that all the animals urinated frequently, and accordingly experiments were carried out to study this action more carefully. An increased flow of urine was to be expected, being due to the salt action. In order to eliminate this factor I compared its effect with that of sodium chloride making both solutions isotonic with the blood.

2-7-10. Rabbit, Weight 2.1 Kg. Anaesthetic: paraldehyde (1.7 per Kg.) per stomach. Bladder cannula inserted. Drug injected into external jugular vein

TIME	AMOUNT OF URINE	REMARKS
2:10		
:15	0.260 G.	
:20420	
:25420	
:30370	
:35310	{ 10 cc. Sodium Camphorate $\Delta = 0.58$ Required some ether to allow of injection.
:40340	
:45450	
:50320	
:55230	
3:00350	
:05290	
:10290	10 cc. NaCl. $\Delta = 0.58$ Few whiffs of ether given as before.
:15300	
:20510	
:25260	
:30470	
:35490	
:40600	
:45320	
:50410	
:55430	
4:00260	
:05400	
:10540	
:15430	
:20260	
:25460	
Discontinued		

The above experiment showed that any diuretic effect which the drug possessed was due directly to salt action.

ACTION UPON THE CIRCULATION

The effect upon the circulation was studied in the cat, rabbit and dog. The result in a general way was the same in all these animals, namely to produce a small but constant rise in pressure with either small or large doses. The extent of rise was greatly influenced by the depth of anaesthesia. In some cases, especially with the rabbit, large doses would invariably give a preliminary fall followed by the usual rise. A sustained fall would also be caused by small or large doses given late in the course of

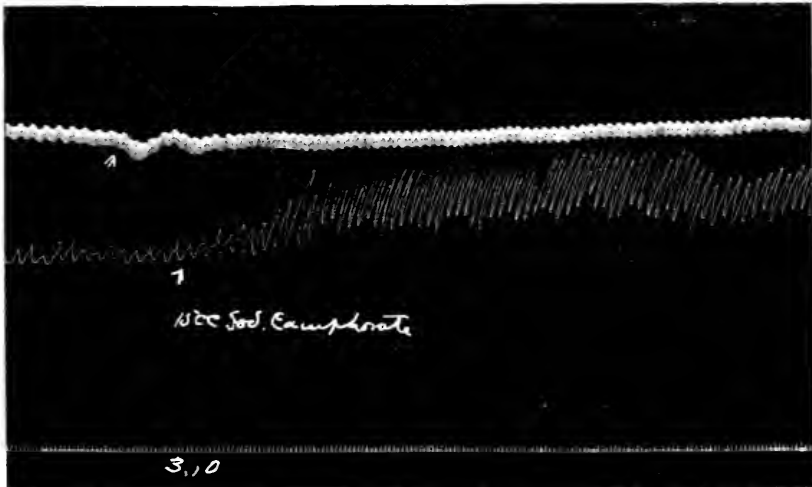


FIG. 1. Effect of sodium camphorate solution, isotonic with the blood, in a cat of medium size. Upper tracing shows the carotid blood pressure and the lower respiratory movements.

the experiment when the animal already had had large amounts of the drug. Both the preliminary fall and the sustained fall in pressure described above as well as the subsequent rise in pressure are due to salt action as was shown by the fact that when isotonic solutions of sodium camphorate and sodium chloride were injected into the blood, similar circulatory changes were produced. The heart rate was decreased in every experiment but one, the slowing being the same after atropine as before.

The following protocol shows the effect of small and large doses of sodium camphorate in a lightly anaesthetized cat while the curves of Fig. 1 taken from another animal represent the effect of an isotonic solution of sodium camphorate upon the blood pressure and respiration.

Blood Pressure. Cat. 1-7-10. Weight 2.6 Kg. Anaesthetic 0.2 G. chloretone per Kg. and ether. Vagi cut

TIME	HEART RATE	RESPIRATION	BLOOD PRESSURE	EXTENT OF RESPIRATION CURVE	REMARKS
	<i>per 20 sec.</i>	<i>per 20 sec.</i>	<i>mm.</i>	<i>mm.</i>	
3-8'-10"	64	7	64	30	0.05G. sodium camphorate per Kg.
9'.....	63	5	76	55	
10'.....	65	6	70	40	
13'.....	65	7	74	27	
21'.....	61	7	70	34	
21'-40"....	60	7	70	32	0.2G. sodium camphorate per Kg.
22'.....	58	6.5	90	45	
-20"....	62	6	100	43	
23'-20"....	63	7	100	38	
26'.....	61	7	90	30	
27'-20"....	?	7	80	29	0.4G. sodium camphorate per Kg.
30'-40"....	63	7	86	28	
31'.....	?	7	98	40	
32'.....	?	7	120	60	
34'.....	?	7	110	28	
37'.....	?	7	100	30	
Discontinued					

ACTION ON SWEAT GLANDS

The sciatics of cats and dogs were exposed and sweating produced experimentally by stimulating the nerve trunk with the electric current. Sodium camphorate was then injected but in no case did it have any effect upon the secretion of sweat. Sweating was obtained in the dog after 16 grams of sodium camphorate had been given and in the cat after 5.2 grams. The drug then has no effect upon peripheral nerve endings.

ACTION UPON RESPIRATION

As a respiratory stimulant sodium camphorate has a distinct effect upon the higher animals as shown by an increase in the depth of respirations, and in the volume of air expired. The extent of respirations was found by using a tambour and piston recorder, while the respiratory volume was measured by means of an air-tight water tank according to the method described by Impens.¹ In brief this is simply a measurement of the volume of expired air by water displacement from an air tight tank. Volume readings were taken for a period of one minute.

Respiratory Volume. 4-6-10: Cat, Weight 2.35 Kg. Anaesthetic, chloretone 0.3 gram per Kg. (per stomach). Vagi cut

TIME	AMOUNT H ₂ O DISPLACED	REMARKS
	cc.	
2:37- :38.....	450	0.05 gram sodium camphorate per Kg.
:40		
:41- :42.....	500	
:44- :45.....	460	0.2 gram sodium camphorate per Kg.
:48		
:49- :50.....	460	
:50- :51.....	480	
:55- :56.....	450	
:59		0.4 gram sodium camphorate per Kg.
3:00-3:01.....	430	
:02- :03.....	510	
:06- :07.....	470	5 cc. sodium camphorate. (Isotonic with blood.)
:14- :15.....	460	
:17		
:19- :20.....	502	
:21- :22.....	470	
:24		5 cc. sodium chloride. (Isotonic with blood.)
:25- :26.....	430	
:27- :28.....	465	
:45- :46.....	430	

¹Impens:—Arch. f. d. ges. Physiol. 1899, lxxviii, 537.

By both methods a distinct increase in respiration was shown. The curve shown on p. 11 the protocol from the cat used 1-7-10, and the above protocol will illustrate this plainly.

DISCUSSION

A study of the pharmacological action of camphoric acid shows that most of its effects are due to salt action. The only well marked physiological action it possesses is its stimulant action on the central nervous system. The clonic convulsions and the increase in respiration would seem to point to an action on the medulla and adjacent areas, resembling in this respect its parent substance camphor.

Clinically camphoric acid has been used extensively to allay sweats incident to phthisis. Any value it may possess in this direction can not be ascribed to a paralyzing action upon the nerve ends in the sweat glands such as is produced by atropine, as camphoric acid is utterly devoid of any such property.

According to Kobert these sweats are asphyxial in origin, being due to a depression of the respiratory center. Therefore the stimulant action of camphoric acid upon the respiratory center would serve to explain the favorable results reported in this disease. Similar good results have been reported from the use of other medullary stimulants such as picrotoxin.

CONCLUSIONS

1. Both on frogs and higher animals, sodium camphorate acts as a stimulant to the central nervous system, producing in the frog at times a distinct increase in reflexes.

2. It is devoid of effect upon the secretory nerve endings of the sweat glands and in addition has no influence on the salivary secretions.

3. The drug given as the sodium salt is comparatively non-toxic for frogs, cats or rabbits. It is more toxic when given intravenously than subcutaneously.

4. Its diuretic effects are comparable to those of an indifferent salt such as sodium chloride.

5. It raises the blood pressure in mammals and has some depressing effect upon the heart muscle. These circulatory changes like the effects upon the kidney are due to salt action.

6. Camphoric acid given to animals as sodium camphorate is a true respiratory stimulant.

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ON THE INFLUENCE OF VARIOUS SALTS UPON TETANY FOLLOWING PARATHYROIDECTOMY

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In a previous publication,¹ after studying the tetany produced by parathyroidectomy, we pointed out that it was possible to cure the symptoms of tetany by the intravenous injection of a solution of a calcium salt. Previous work upon the tetany produced in this way had led one of us to the conclusion that, in all probability, the symptoms were produced by some circulating poison formed in the process of metabolism; but this poison could not then be demonstrated by any test, and even yet no definite demonstration of its presence has been brought forward even by the most obvious biological tests. In view of the remarkable effect of the injection of calcium salts, which bring about almost instantaneously a cessation of the symptoms, we thought that it might be possible that the symptoms of muscular twitching, etc., were due to a lack of the usual amount of calcium in the nerve cells; for it has been shown by numerous investigators, as we pointed out in that paper, that the removal, by precipitation, of the normal calcium of the tissues produces a condition of irritability in those tissues which is followed by muscular twitchings.

We expressed an idea, therefore, that possibly the parathyroid normally controlled, in some way, the amount of calcium in the tissues and the interchange of calcium in the course of metabolism in these cells, so that the loss of the parathyroid secretion would allow of a disturbance in this calcium metabolism in the

¹ MacCallum and Voegtlin: Jour. Exp. Med., 1909, xi, 118.

sense that the cells become impoverished with regard to calcium, and consequently become more irritable.

We attempted to show that this was the case by the investigation of the metabolism of animals thrown into tetany by parathyroidectomy, and chemical studies were made by one of us (Voegtlin) of the excreta and of the blood and brain of such animals. He found a somewhat increased output of calcium in the urine and feces, and much more definitely, a decrease in the content of calcium in the blood and brain. Since this time he has made further estimations of the quantities of calcium contained in the blood and in the brain of parathyroidectomized animals suffering from tetany, and still finds quite regularly, a diminution in the amount of calcium.

The well-known irregularity in the excretion of calcium in normal animals, and the slight variations which we actually found in the amount excreted in the tetany animals, as contrasted with the normal, has led us to practically abandon this method of investigation as of little value. The investigation of the calcium content of the blood and brain, however, seems to be of more value, and the objection that this is the estimation of one inorganic substance alone without regard to the relative amounts of the other inorganic substances seems not to weaken the suggestiveness of this association. Nevertheless, we must admit that in such studies we have not any absolute proof that the symptoms of tetany are dependent solely upon the loss of the normal proportion of calcium in the tissues.

In the paper quoted, we have referred to the results of previous papers as follows:

"We find no other explanation of this phenomenon than that some poisonous material, not destroyed or present in larger amounts than physiologically, on account of the absence of the parathyroid glands is circulating in the blood. We could not demonstrate the presence of this poisonous substance by injecting the blood from dogs in tetany into normal animals but, it may readily be imagined that it is some substance easily oxidized or otherwise changed into harmless materials in the normal body, but circulating in the parathyroidectomized dog on

account of the lack of some ferment-like material produced normally by the parathyroid. The idea of a circulatory poison we have kept in mind throughout our subsequent experiments.²⁷

Since the appearance of this paper much work has been done upon the subject by many investigators, of whom every one recognizes the fact that tetany can be quickly, though temporarily, cured by injections of calcium, although many of the recent papers have concerned themselves with the attempt to lessen the importance of this fact by pointing out that similar results may be obtained with a variety of other drugs, most of which were studied and reported on in that paper.

Our present experimental work has been carried on with the idea of determining the precise mode of action of these inorganic substances and of deciding, if possible, whether their effects throw any light upon the real nature of the tetany itself. It may be stated briefly that we have been more and more led back to the original idea and believe that no matter what the part played by the calcium, we are probably dealing with a condition in which some poisonous material is developed in the course of metabolism, deprived as it is of the influence of the parathyroid; and that with our large doses of calcium, we mask the effect of this substance upon the nerve cells. Whether this poisonous substance acts by reducing the calcium content of the nerve cells seems still perfectly possible and not for a moment to be left out of consideration.

The further study of the nature of this hypothetical poison, we reserve for another paper, and at present concern ourselves only with the study of the effects of the inorganic and other substances which have been used in the work on tetany, comparing their effect on parathyroidectomized and normal animals.

We have, since our previous work, adopted the use of a galvanic battery in testing the electrical excitability of the nerves, and in each experiment changes in the electrical excitability are recorded, which, in many instances, allow us to recognize the existence of

² Loc cit.: p 129, referring to MacCallum and Davidson, *Medical News*, 1905, lxxxvi, 625.

tetany, and throw much more light upon the character of the results of injections than the mere observation of the symptoms. For this purpose we have used a dry cell battery fitted with a switch to reverse the poles, and a rheostat and Malaquin milliammeter, reading to tenths of milliampères. One of the electrodes used is an indifferent, sponge-covered brass electrode measuring 5 cm. in diameter, which is placed on the abdominal wall on the right side just above the groin. The other, the stimulating electrode, has a small round point covered with sponge not quite 1 cm. in diameter and is fitted with a current interrupter worked with the thumb. This is applied to the outer side of the right leg just below the knee, in the cleft between the heads of the gastrocnemius directly over the peroneal nerve which can be felt and rolled under the finger. The minimum current sufficient to give a visible upward jerk to the foot was recorded. Well known differences exist between the current required to give a contraction with the cathode opening and closing shocks and the anode opening and anode closing shocks and in each instance in which it proved to be impossible to determine at what point the cathode opening contraction appeared, the point at which cathode closing tetanus was produced was recorded.

EXPERIMENTS WITH CALCIUM

Of the experiments with calcium salts, we may refer to ten which have been described in some detail in our previous paper: Nos. 2107, 2207, 108, 2208, 2508, 3708, 6908. These experiments show very clearly the beneficial action of calcium injections upon the course of tetany. Even when given by mouth, as in the last three, the effect was very good, and these dogs were kept alive a long time in this way. We wish to lay some emphasis upon this point, inasmuch as several recent writers have made the statement that it is perfectly impossible to keep alive, by medication dogs from which the parathyroids have been removed.

In the early experiments, the doses of calcium were given by injecting the solution into a vein from a syringe rather than from a burette. In all probability, they were scarcely sufficient to

produce the most complete abolition of tetany, for in later experiments, in which the calcium was allowed to run in until tetany had disappeared, the results have been much more conclusive. The amounts used will be stated in each protocol.

1115. January 17. Dog, weight 7 kg. Complete thyro-parathyroidectomy.

January 18. Well enough.

January 19. 2 p.m.—Slight tetanic twitchings.

January 20. 2 p.m.—Marked tetany. 2.40—distinct twitchings, rapid breathing; in the most violent tetany. KC-0.8; KO-2.2; AC-1.6; AO-1.6. Pulse 150, respiration 28. 2.50 p.m.—10 cc. $\frac{M}{8}$ calcium lactate run in. 2.55 p.m.—KC-3.0; KO-negative at 7.0; AC-2.4; AO-5.2. 3.00 p.m.—10 cc. run in. 3.07 p.m.—20 cc. run in. In all 45 cc. $\frac{M}{8}$ calcium lactate introduced. Dog struggles a good deal.

KC-4.0; KO-negative at 7-; AC-4.8; AO-negative at 9 plus. 3.20 p.m.—No signs of tetany. Pulse 54, respiration 24. 4.20 p.m.—Dog all right. KC-2.0; KO-negative; AC-3.0; AO-negative at 8 plus. 10.30 p.m.—Dog is well and lively. Slight fibrillary tremors of the tongue. KC-1.7; KO-negative at 8.0; AC-2.6; AO-3.8 to 4.0.

January 21. 5.35 p.m.—Slight fibrillary tremors of tongue. KC-0.8; KO-1.7; AC-0.8; AO-1.7. On account of absence from the laboratory the dog was not further observed. He died some days later.

1124. January 18. Dog, weight 8.8 kg. Complete thyro-parathyroidectomy.

January 20. Dog in violent tetany. Tachypnea, snapping of jaws, etc. 11.30 a.m.—KC-0.2; KO-1.2; AC-0.3; AO-2.2. 11.45 a.m.— $\frac{M}{8}$ calcium lactate run into jugular. From 11.45 to 11.50—16 cc. 11.50 a.m.—KC-0.3; KO-5.0; AC-0.9; AO-2.9. From 11.55 a.m. to 12.00—34 cc. run in. At 11.58—Dog still has slight twitching. Pulse 92, respiration 44. At 12.00 noon, 50 cc. calcium solution in all had been run in. KC-0.8; KO-negative at 8.0; AC-3.0; AO-positive at 8.0.

This shows the remarkable effect of calcium in raising the threshold of electrical excitability and relieving tetany.

12.10—Dog is perfectly quiet. Walks about. Shivers slightly but tremors of tongue are gone. 1.00 p.m.—Pulse rapid, 210; respiration 24. KC-1.2; KO-negative at 8.0; AC-3.8; AO-5.8. At 5.00 p.m.—Slight fibrillary tremors of tongue. Otherwise dog is perfectly comfortable and quiet. Pulse 136, respiration 40. KC-0.9; KO-1.2; AC-0.6; AO-1.0.

January 21. 5.00 p.m.—KC-less than 0.1; KO-0.7; AC-0.1; AO-1.2. Respiration 42, labored. Pulse, 180. Marked tetanic twitchings. It is seen from this that during the tetany the KC sinks to such a point that it cannot be measured by this instrument. 5.10 p.m.— $\frac{M}{8}$ calcium lactate run into the jugular vein. From 5.14 to 5.16—40 cc. run in. During this time the tetany came to an abrupt stop. 5.20 p.m.—KC-0.6; KO-5.0; AC-1.3; AO-negative at 9 plus.

January 22. Dog was found all right in the morning. At 6.00 p.m. was in violent tetany; twitching of all the muscles. Respiration 54, very labored; pulse 192, rather weak. KC-0.05; KO-0.07; AC-0.10; AO-0.6. At 6.15 p.m.—50 cc. $\frac{M}{8}$ calcium lactate run into jugular vein during violent twitching and grinding of teeth. Pulse sank to 80 and became extremely irregular. 6.18 p.m.—Almost completely relaxed within last three minutes. Pulse 168. Slight twitching persists. 6.20 p.m.—15 cc. more injected into jugular. Pulse 150. Dog is quite relaxed. No tremors. Respiration almost stopped for a time, but at 6.24 is improving. KC-0.9; KO-negative at 8.0; AC-5.0; AO-negative at 10. Dog completely relaxed. Pulse 180. Perfectly helpless and apathetic. This seemed remarkable, for with the injection of calcium we had never found any such complete disability on the part of the animal. The material in the burette was tested and it was found that, through mistake, the last 16 cc. were really from the bottle containing an $\frac{M}{8}$ solution of magnesium sulphate instead of calcium, although the first 50 cc. were really from the solution of calcium lactate. The dog recovered, however, and next day was dull and stupid but able to walk about. Was not observed further but was killed two days later. This experiment emphasizes the anaesthetic and depressing action of magnesium as compared with that of calcium.

1131. February 24. Dog, weight 5.9 kg. Complete thyro-parathyroidectomy.

February 26. Dog in moderately violent tetany with jerkings of legs and twitching of muscles in general. KC-0.05; KO- not determined; AC-0.05; AO- Minimal. 1.25 p.m.—10 cc. $\frac{M}{8}$ calcium chloride run into jugular. Dog became perfectly quiet but some fibrillary twitchings are still to be seen under the skin and in the tongue. 1.33 p.m.—KC-0.05; KO-0.09; AC-0.1; AO-0.6. 1.34 p.m.—5 cc. more run in. Dog perfectly at rest. Still some fibrillary tremors. KC-0.05; KO-1.3; AC-0.2; AO-2.6. 1.40 p.m.—5 cc. more run in. 1.45 p.m.—10 cc. more run in. Pulse 87, irregular and strong. KC-0.05; KO-1.7; AC-0.2; AO-4 plus. 1.50 p.m.—10 cc. more run in making 40 cc. in

all. Dog perfectly quiet. No fibrillary tremors. KC-0.05; KO-4.0; AC-0.5; AO-above 10.

February 27. 10.45 a.m.—Distinct tetany. Respiration 54, labored. KC-below 0.1; KO-0.7; AC-0.1; AO-0.5. 11.06 a.m.—25 cc. $\frac{M}{8}$ calcium lactate run into jugular. Dog became completely relaxed. Respiration easy, 24 to the minute. Pulse 84, strong. 11.10 a.m.—No signs of twitching. KC-below 0.1; KO-5 plus; AC-0.6; AO-4.4. At 11.20 a.m. dog was put on the floor and walked about and was apparently perfectly normal. 4.45 p.m.—Dog is still apparently quite normal. Eats a great deal.

February 28. 12.30—Dog found with distinct twitchings in legs. Jaws clenched. Very marked fibrillary tremors of tongue. No tachypnea. Pulse 156, respiration 48. KC-less than 0.1; KO-0.5; AC-less than 0.1; AO-0.3. 1.15 to 1.17 p.m.—38 cc. $\frac{M}{8}$ calcium chloride run into jugular. 1.20 p.m.—Quite relaxed. Fibrillary tremors have gone. KC-0.4; KO-5 plus; AC-1.1; AO-8 plus.

March 1. 9.30 a.m.—Found in severe tetany. 10.20 a.m.—Respiration, 120, pulse cannot be counted on account of twitchings. Dog lying on side helpless. KC-0.2; KO-0.6; AC-0.4; AO-0.8; 10.30 a.m.—10 cc. $\frac{M}{8}$ calcium lactate run in, after which there was marked improvement. The twitching practically stopped and the dog was completely relaxed. 10.33 a.m.—20 cc. in all had been run in. Pulse 66, very strong and irregular. All twitchings gone. KC-0.2; KO-0.4; AC-1.9; AO-5 plus. When set on floor acted like normal dog.

March 2. 10 a.m.—Tetany observed. 2.00 p.m.—Dog was in violent tetany. KC-less than 0.1; KO-0.6; AC-0.1; AO-0.6. 4 cc. curari solution was injected into the vein. During injection dog went into epileptiform convulsion but was kept alive by artificial respiration. Heart kept on beating vigorously. After the injection of the curari, the electrical excitability of the nerves practically disappeared, as far as could be determined with our instrument, and no contraction of the muscles could be elicited by stimulation of the nerve. 2.55 p.m.—KC-10.0; KO-25 plus; AC-3 plus; AO-25 plus. 4.55 p.m.—Dog had become conscious. Vomited. Taken from the table and walks with some difficulty, but no signs of tetany.

March 3. 11.30 a.m.—Fibrillary twitchings of tongue. Dog is lively and playful. KC-0.1; KO-1.6; AC-0.3; AO-0.6; Pulse 120, regular. Respiration 24.

March 4. No tetany.

March 5. 10 a.m.—Found dead. There was evidently an infection of the operation wound.

1135. February 25. Dog, weight 8 kg. Four parathyroids removed.

February 27. Fibrillary twitchings of tongue.

February 28. Dog shivering, with fibrillary twitchings of tongue. Slight subcutaneous twitching over legs and shoulder and neck. Pulse 144, respiration 16. KC-0.1; KO-2.3; AC-0.1; AO-2.0. Between 3.55 and 4.30 p.m. 150 cc. of distilled water was run into the jugular vein. This experiment was made to determine whether the inclination to tetany could be increased in a highly excitable dog by giving distilled water. Evidently this is not the case, however, for at 4.35 p.m. twitching was not increased, although there was still a general quivering. KC-below 0.1; KO-2.3; AC-0.1; AO-1.1.

March 1. No tetany.

March 2. 4 p.m.—Tetany. Respiration 120. Muscular twitching all over the body. KC-less than 0.1; KO-0.9; AC-0.1; AO-0.6. At 4.10 p.m.— $\frac{M}{8}$ calcium lactate was injected subcutaneously and into the muscles. Between 4.10 and 4.35 p.m. 30 cc. were run in. At 4.35 the dog was much more quiet. Respiration 60. Twitchings still noticeable, however. 4.45 to 5.05 p.m.—40 cc. more was introduced. At 5.05 p.m.—KC-less than 0.1; KO-1.4; AC-0.3; AO-0.8. At 5.30 p.m. all twitching had disappeared. Dog is playful and apparently normal. KC-less than 0.1; KO-2.8; AC-0.3; AO-4.0.

March 3. At 12 noon, no signs of tetany. A large area of oedema, involving the right foreleg and thigh is seen at the site of injection. Heart very strong. Pulse 140, respiration 20. KC-less than 0.1; KO-4.0; AC-0.6; AO-3.1.

March 4. No tetany.

March 5. No tetany. Extensive abscess formation in subcutaneous tissue. The dog was killed with ether and at autopsy the abscess was found to extend down to end of sternum from right shoulder. There was also lobar pneumonia.

1141. February 28. A normal dog, weighing 7.2 kg. was tested under ether. KC-0.6; KO-6.0; AC-1.4; AO-1.2.

March 1. 4 p.m.—Dog normal. KC-0.6; KO-7.2; AC-0.3; AO-2.8. Pulse 150, respiration 24. 4.15 p.m.— $\frac{M}{8}$ calcium lactate run into jugular. After a few cc. the heart beats became irregular. By 4.20 p.m. 30 cc. had been run in. Pulse 66, very strong. Respiration 28, with expiratory spasm. KC-0.6; KO-10 plus; AC-0.8; AO-2.8. 4.30 p.m.—Dog vomited yellow fluid.

March 2. 10 a.m.—KC-1.6; KO-10 plus; AC-1.2; AO-4.6. Pulse 120, respiration 16.

March 3. 3.20 p.m.—Pulse 160, respiration 20. KC-0.1; KO-tetanus at 5.5; AC-1.6; AO-2.4.

From these experiments it is seen that the calcium injections both in normal dogs and in those with tetany, has an overwhelming effect in dulling the excitability of the motor nerves to electrical stimulation. The change produced is particularly marked with respect to the cathode and anode opening shocks, but it is also striking with respect to the others. The rapidity with which this effect appears and the absence of ill effects should be especially observed. The results of experiment No. 1135, however, may be taken as a warning against the subcutaneous injection of calcium salts, which is likely to be followed by necrosis of the tissue and inflammation.

EXPERIMENTS WITH MAGNESIUM

In our previous paper we have recorded the beneficial effects of magnesium chloride given intravenously in two cases and have said that these two experiments seemed to indicate that the injection of magnesium will suppress tetany, though its effects are somewhat confused by the toxic action of the magnesium itself. For, whereas dog No. 1508 was extremely stuporous the next day, other dogs suffering equally severely from tetany at the same time, and which had received an injection of calcium salt, were not only without twitching the next day but were also perfectly bright and well. Our present experiments confirm these results, and one has already been recorded (1124).

1117. January 18. Dog, weight 9.9 kg. Complete thyro-parathyroidectomy.

January 19. No symptoms.

January 20. No symptoms.

January 21. 11 a.m.—Found in severe tetany. 12.15—KC-0.1; KO-0.8; AC-0.1; AO-1.0. Respiration 108, pulse 138. 12.30 p.m.— $\frac{3}{8}$ magnesium sulphate run into jugular. 12.31 p.m.—12 cc. had run in. Animal relaxed. Twitchings less pronounced. 12.37 p.m.

—20 cc. had run in. Respirations much slower and deeper, 20 per minute. 12.40 p.m.—30 cc. had run in. Tongue tremors still present. Other twitchings gone. 12.45 p.m.—40 cc. had run in. 12.50 p.m.—Pulse 120, respiration 16, very deep. KC-0.1; KO-negative at 8 plus; AC-0.6; AO-3.1. 2.20 p.m.—No signs of tetany. Respiration 30. Pulse 152. KC-0.2; KO-1.8; AC-0.5; AO-4.2. 5.30 p.m.—No signs of tetany. KC-0.1; KO-1.8; AC-1.2; AO-4.2. Dog showed only slight twitchings for two or three days after this, when he was found dead.

1120. January 18. Dog, weight 8.9 kg. Complete thyro-parathyroidectomy.

January 19. Shows no symptoms.

January 20. 1 p.m.—Is apparently normal. At 3.25—Dog found in violent tetany. Respiration 160. 3.38 p.m.—KC-0.2; KO-0.6; AC-0.2; AO-0.6. 3.47–3.57—50 cc. $\frac{M}{8}$ magnesium sulphate run into jugular. At the end of this time the dog became quite relaxed although twitchings were still present. Respiration 160. KC-0.2; KO-negative at 8 plus; AC-1.0; AO-1.0. 4.12 p.m.—Pulse 120, respiration 124. No twitchings. Dog seems perfectly normal. 5.10 p.m.—No change in general condition. KC-0.2; KO-negative at 8 plus; AC-1.0; AO-2.4. 10 p.m. KC-0.2; KO-5.8; AC-1.3; AO-3.0. Dog is well and quiet. Pulse 120, respiration 16. Tongue shows slight tremor but these twitchings are scarcely visible.

January 21. 3.30 p.m.—KC-0.1; KO-negative at 8 plus; AC-0.8; AO-0.5. Definite tongue tremor but no twitching of muscles. Pulse 126, very strong. Respiration 30.

January 22. 1.15 p.m.—Dog found in violent tetany with marked tachypnea. Respiration 222. KC-0.01; KO-0.8; AC-0.2; AO-0.4. 1.30 p.m.—Pulse 180. Respiration 72. Grinds his teeth. $\frac{M}{8}$ magnesium sulphate started to run into jugular. 1.33 p.m.—10 cc. had run in. The dog improved at once. Was very quiet. Heart slower and stronger, 144. 1.33 to 1.40 p.m.—20 cc. run in. Twitchings not all gone. KC-0.01; KO-0.5; AC-0.02; AO- . At 1.45—50 cc. in all had been run in. Fibrillary tremors disappeared. Dog quite apathetic and relaxed. 2.30 p.m.—Walks about as though drunk. No fibrillary tremors. No twitchings. Can walk but is apathetic.

January 24. Dog dull and weak. No twitchings. Refused to eat. On January 25 found dead.

1138. February 25. Dog, weight 10.8 kg. Complete thyro-parathyroidectomy.

February 26. Found in violent tetany. KC-0.01; KO-0.35; AC-

0.1; AO-0.3. At 6.30 p.m. $\frac{M}{8}$ magnesium chloride started into jugular. Between 6.30 and 6.40—50 cc. of the solution was run in. After 26 cc. pulse was 150. After 30 cc. dog is quiet, respiration very shallow. The solution was probably going in a little too fast. Respiration sank to 42. Twitching almost entirely gone but trace still exists in the shoulder and legs. Respiration 168. 6.40 p.m.—KC-0.9; KO-3.4; AC-2.0; AO-2.0. 10 p.m.—Dog found lying quiet. Very dull but apparently comfortable. 11.35 p.m.—Apathetic and quiet. Absolutely no twitching. 11.45 p.m.—KC-0.7; KO-above 5; AC-1.0; AO-0.6.

February 27. 10 a.m.—No tetany. 2.45 p.m.—Distinct tetany. Respiration 44, pulse 120. Heart beat can be noticed over abdomen. KC-0.1; KO-tetanus at 1.0; AC-0.6; AO-0.8. 3 p.m.—24 cc. $\frac{M}{8}$ magnesium chloride run in within 8 minutes. Tetany disappeared. KC-0.6; KO-tetanus at 2.0; AC-2.0; AO-1.0. 3.15 p.m.—Dog is perfectly comfortable. 4.45 p.m.—Dog looks depressed but there are no signs of tetany.

February 28. 10 a.m.—Slight tetany. Twitchings of muscles of anterior part of body. 4.55 p.m.—Still in slight tetany. KC- below 0.1; KO-0.8; AC-0.4; AO-0.4. Pulse 120, respiration 16. Heart beat noticed over abdomen. Slight twitching of tongue and muscles. 5.05 p.m.—17 cc. $\frac{M}{8}$ magnesium chloride run in. All symptoms stopped. 5.07 p.m.—KC-0.2; KO-1.2; AC-0.4; AO-0.6. 5.30 p.m.—Seems perfectly normal. (The electrical test in this instance was made, as in many other instances, much too soon after the injection of the salt to show the full effect on the electrical excitability. This, however, is corrected in the remainder of this experiment.)

March 1. 11 a.m.—Distinct tetany. Twitching all over body. Pulse 132, very strong. Heart beat plainly seen. Respiration 30. Pupils contracted. KC-less than 0.1; KO-0.4; AC-0.3; AO-0.2. 11.05 a.m.—12 cc. $\frac{M}{8}$ magnesium chloride run in. 11.10—All signs of tetany disappeared. Respiration 16, pulse 124. Pupils contracted. KC-0.1; KO-0.8; AC-0.4; AO-0.4. Dog seems somewhat depressed. 11.20 to 11.22 a.m.—30 cc. $\frac{M}{8}$ magnesium chloride run in. Dog is found to be absolutely anaesthetic to pain. Pupil reacts to light. 11.33 a.m.—50 cc. in all had been introduced. Eye reflexes quite gone. KC-1.2; KO-8 plus; AC-6.0; AO-4.0. Blood is highly venous with signs of asphyxia. Dog completely anaesthetic. Respiration 24. From 11.52 to 11.59 a.m.—40 cc. $\frac{M}{8}$ calcium chloride run in. Heart rate and respiration increased. Eye reflexes returned. KC-0.6; KO-8 plus; AC-2.0; AO-1.8. 12.10—Dog looks perfectly normal.

March 2. No tetany. Has beginning pneumonia.

March 3. Dead.

1142. March 3. Normal dog, weight 9.8 kg. 11 a.m.—KC-0.2; KO-12 plus; AC-1.2; AO-2.4. 11.25 to 11.30—50 cc. $\frac{M}{8}$ magnesium chloride run into jugular vein. 11.32—Pulse 114, respiration 16. 11.35—KC-0.2; KO-12 plus; AC-2.6; AO-9.0. No change in general appearance. Dog is still lively. 3.30 p.m.—Pulse 90, respiration 30. KC-0.2; KO-6.0; AC-2.7; AO-5.5. Dog seems practically normal.

March 6. 2.50 p.m.—Dog seems normal. KC-0.4; KO-not found tetanus at 9; AC-0.8; AO-3.2. Pulse 168, respiration 36. 3.15 to 3.30—30 cc. magnesium chloride run into jugular. Pulse 180, respiration 36. KC-0.4; KO-not found; tetanus at 6-10; AC-2.2; AO-3.8. 3.45 p.m.—34 cc. more run in. KC-0.5; KO-not found; AC-1.8; AO-7.0. 4.10 to 4.20 p.m.—50 cc. more run in. Dog rather relaxed. Eye reflex active. Pupil dilated. 4.25 to 4.30—Respiration 48, pulse 125. 18 cc. more run in. Dog quite relaxed. In all 102 cc. of the solution was run into the vein. 4.40 p.m.—5 p.m.—50 cc. $\frac{M}{8}$ calcium chloride was run into the vein. Pulse 150, Dog shivers somewhat. KC-0.9; KO-not found tetanus at 20. AC-4.6; AO-not found. No tetanus at 20. Dog wakes up and becomes tense and struggles. 4.55 p.m.—50 cc. $\frac{M}{8}$ calcium chloride run in in 5 minutes. In all 100 cc. has been introduced. Pupils much smaller, dog has become bright. KC-2.4; KO-not found at 25 and no tetanus; AC-8.0; AO-no tetanus at 25.

1154. March 13. Normal dog, weight 7.7 kg. At 4 p.m.—KC-0.6; KO-tetanus at 5.0; AC-6.0; AO-7.0. 4.10 to 4.18 p.m.—12 cc. $\frac{M}{8}$ magnesium chloride run into jugular. After 5 cc., pulse 204. Dog licks his lips. Respiration 72. At 7 cc. vomited. Reflexes still good. Is quiet. Pulse 180, strong. Respiration 54. Vomited again at 12 cc. From 4.18 to 4.25 p.m.—Second 12 cc. were run in. Respiration spasmodic. Dog rather quiet but not entirely anaesthetic. Eye reflex present. Pulse 150. Respiration slow, 42. At 4.30 p.m.—KC-1.9; KO-tetanus at 5.6; AC-5 to 6; AO-tetanus at 20. From 4.50 to 4.55—20 cc. more run in. In all, the dog has received 44 cc. Urinates. Breathing shallow and slow. Pulse 144, running. Dog is apathetic but not cyanotic. Reflexes inactive. KC-4.0; KO-tetanus at 25.0; AC-9.0 AO-25.0, not definitely found then. Dog is sensitive to this current. From 5.00 to 5.10—40 cc. $\frac{M}{8}$ calcium chloride run into jugular. KC-6.8; KO-tetanus at 25.0; AC-10.0; AO-not determined.

March 16. 4 p.m.—Dog is well. KC-0.8; KO-tetanus at 8.0; AC-2.7; AO-6.5.

1155. March 16. Normal dog, weight 5.1 kg. KC-0.3; KO-tetanus at 2.3; AC-0.6; AO-1.0. 4.50 to 5.00 p.m.—4.5 cc. $\frac{M}{2}$ magnesium chloride run into the vein. Pulse 181. Dog is very quiet. Shivers. Respiration 12. Inspiration much prolonged. Pulse irregular, slowing down to 144. At 5.03 p.m.—7 cc. more run in. Dog is quite relaxed. Respiration labored and hurried. Reflexes gone. At 5.08 p.m.—Dog having received 11.5 cc. in all is quite anaesthetic, but reflexes are returning. Respiration sighing. KC-0.2; KO-tetanus at 2.2; AC-0.6; AO-1.4. At 5.12 p.m. $\frac{M}{2}$ magnesium chloride run in up to 15 cc. Completely anaesthetized. Pulse 126, respiration 30. Lid reflexes still present. KC-0.4; KO-tetanus at 4+; AC-1.4; AO-10.0. 5.15 p.m.—Up to 20 cc. run in. Reflexes are abolished. Pulse 108, respiration 10. Dog is apparently relaxed. Only the slightest reflex. At 5.25 p.m.—KC-0.3; KO-tetanus at 10; AC-1.6; AO-tetanus at 16.5. 5.27 to 5.32 p.m.—40 cc. $\frac{M}{8}$ calcium lactate run into jugular. Dog begins to shiver at once and from being perfectly relaxed becomes tense and quivering, and groans. Eye reflexes active. At 5.35 p.m.—KC-2.0; KO-tetanus at 7.2; AC-1.5; AO-20. At 5.40 p.m.—Can walk about. Urinated abundantly. 6.10 p.m.—KC-0.9; KO-positive at 7.5; AC-1.6; AO-positive at 4.0.

From these experiments it is evident that the symptoms of tetany can be stopped rapidly by the injection of magnesium, and that the magnesium acts essentially by diminishing the electrical excitability of the motor nerve. That it has some other action, however, is shown by the anaesthetization, by the loss of reflexes and also by the fact that an injection of calcium antagonizes its effects in this sense, as has been shown by Meltzer. It is perfectly clear from these further experiments, that, as we pointed out in our previous paper, there are certain practical objections to the use of magnesium for the mere curing of tetany inasmuch as it leaves the animal in a stuporous and very much depressed condition.

EXPERIMENTS WITH STRONTIUM

The following experiments were carried out with strontium salts.

117. January 16. Dog, weight 4.4 kg. 2 parathyroids from right and whole thyroid lobe removed from the left side.

January 17. 8 p.m.—Slight twitching.

January 19. Dog found in violent tetany, helpless rigidity and tachypnea. 11.44 a.m.—Respiration 180, pulse 174. 11.45 to 11.49—25 cc. $\frac{M}{8}$ strontium chloride run into jugular vein. Respiration during this, 220. 11.53 a.m.—Pulse 90, respiration 114. No twitchings. Dog lies quiet, but occasional slight twitchings. 11.56—Quiet. Lies totally relaxed. Slight twitching in cheek. 12.00—No signs of twitching. The effect of the strontium is very distinct. 2 p.m.—No sign of tetany. Pulse 84, respiration 16. Animal looks normal.

January 20. 1 p.m.—Dog seems well. 5.20—Found in distinct tetany. Respiration 24. KC-below 0.1; KO-0.6; AC-0.1; AO-0.6. 8.45 p.m.—Found in violent tetany. Respiration 200. 9.15 to 9.20 p.m.—54 cc. $\frac{M}{8}$ strontium chloride run in. Twitching stopped at once but dog still showed tachypnea. 9.25 p.m.—Tachypnea still persists. Pulse 150, strong. KC-0.1; KO-0.1; AC-0.1; AO-1.6. The strontium has evidently stopped the twitching but has not sufficiently lowered the excitability. 9.40 p.m.—Dog is apparently all right. Grunts a little but walks about. 12.45 p.m.—Quiet. KC-less than 0.1; KO-5.2; AC-1.0; AO-3.8.

January 21. No tetany. 3.50 p.m.—Pulse 120, respiration 12. Dog eats but looks rather depressed. KC-less than 0.1; KO-8 plus; AC-1.2; AO-6 plus.

January 22. Dog is fairly well. Slight twitching of tongue. KC-0.05; KO-1.0; AC-0.05; AO-2.0. After this showed no symptoms of tetany but became emaciated and died January 31.

1113. January 17. Dog, weight 7.1 kg. 2 parathyroids removed from right side, whole lobe from left side.

January 18. Well enough.

January 19. Violent tetany. Pulse 110, respiration 42. 4.52–4.58 p.m.—31 cc. $\frac{M}{8}$ strontium chloride run into vein. Twitching stopped immediately. 5.05 p.m.—Dog apparently normal. Playful. Drinks water. 5.10 p.m.—Eats meat with great appetite.

January 20. Well at 10 a.m. 11.20 a.m.—Having quite violent twitchings. KC-0.2; KO-not determined; AC-2.2; AO-2.2.

January 21. 4.15 p.m.—Pulse 150, respiration 72. Some muscular twitchings. Seems otherwise quite well. KC-0.1; KO-0.4; AC-0.6; AO-0.3. 4.41 to 4.46 p.m.—40 cc. $\frac{M}{8}$ strontium chloride run in. All signs of tetany disappeared. At 4.50 p.m.—KC-0.6; KO-negative at 8 plus; AC-2.5; AO-1.6. Pulse 120, respiration 42. Dog not observed after this. Was found dead on January 26.

1132. February 24. Dog, weight 6 kg. Total thyro-parathyroid-ectomy.

February 26. Found in violent tetany. Fibrillary tremors of tongue KC-below 0.1; KO-tetanus at 0.8; AC-below 0.1; AO-0.9. No excessive muscular activity. Pulse 72, respiration 34, quiet. 4 to 4.05 p.m.—50 cc. $\frac{M}{8}$ strontium chloride run into jugular. Twitching becomes far less frequent and less distinct. At 4.10 still slight twitchings. KC-below 0.1; KO-2.2; AC-0.2; AO-0.8. 4.20 p.m.—Very quiet and apathetic. Twitching stopped. Fibrillary tremors no longer visible. Pulse 54. 10. p.m.—Found dull and apathetic, but quiet and can walk about well.

February 27. 4.30; p.m.—Slight tetany. KC-below 0.1; KO-0.6; AC-0.2; AO-0.8. 4.35 p.m.— $\frac{M}{8}$ strontium chloride run in. 4.40—20 cc. in; all signs of tetany gone. 4.45—KC below 0.1; KO-5.0+AC-0.2; AO-3.0. Eats with great appetite.

February 28. 10 a.m.—Slight muscular twitchings. Respiration 16, pulse 72, weak. Looks very depressed. KC-below 0.1; KO-1.0; AC-0.1; AO-0.8. 10.25 to 10.30. a.m.—20 cc. $\frac{M}{8}$ strontium chloride run into vein. Tetany stopped at once. 10.35 a.m.—KC-below 0.1; KO-5 plus; AC-0.4; AO-0.9. 10.40 a.m.—Seems normal.

March 1. 12.00 noon—Beginning to have distinct tetany. Muscular twitchings all over body. Respiration 16, pulse 84. Lies on side taking no interest in surroundings. KC-less than 0.1; KO-0.5; AC-0.1; AO-0.6. From 12.30 to 12.33—30 cc. $\frac{M}{8}$ strontium chloride run in. Tetany practically disappeared at once. From 12.33 to 12.38—20 cc. more run in. In all 50 cc. The excessive amount of strontium was used in the hope of preventing the early recurrence of symptoms. KC-less than 0.1; KO-2.6; AC-0.7; AO-2.0. Dog seems perfectly normal.

March 2. 5.15 p.m.—Was well until now. At present shows tremors of the tongue and spasmodic labored respiration. From 5.20 to 5.27—50 cc. $\frac{M}{8}$ strontium chloride run into vein. Tetany disappeared after 20 cc. had been given, and the dog, after all was injected, assumed a normal appearance.

March 3. 12.10 p.m.—Showed occasional fibrillary twitchings of muscles of shoulder and head. 12.35 p.m.—KC-0.1; KO- not found tetanus at 1.6; AC-0.4; AO-0.9. Pulse 72, respiration 24, labored. There are no further notes on this dog except that he was dead on March 5.

1143. March 2. Normal dog, weight 5 kg. 12.40—KC-less than 0.1; KO-10 plus; AC-1.8; AO-12.0. At 1 p.m.—30 cc. $\frac{M}{8}$ strontium chloride run into jugular. Pulse becomes irregular. At 1.05 p.m.—KC-0.2;

KO-tetanus at 9.0; AC-0.7; AO-4.4. 3.50 p.m.—Dog is quite active and well. KC-0.1; KO-tetanus at 9.0; AC-1.7; AO-5.0.

1151. March 8. Dog, weight 6.4 kg. Previously used two days before for experiment with ammonium chloride. Now apparently normal. 11.35 a.m.—KC-0.3; KO-tetanus at 3.2; AC-1.4; AO-3.2. Pulse 138. 11.50 to 12.00—50 cc. $\frac{M}{8}$ strontium chloride run into jugular. At 12.00—KC-0.7; KO-tetanus at 4.8; AC-1.8; AO-3.2; 12.15—Pulse 104, irregular. KC-0.8; KO-tetanus at 6+; AC-2.6; AO-10.0.

On the whole, the results obtained by the injection of strontium salts are similar to those produced by calcium although the lowering of the excitability of the motor nerves is not quite so marked.

EXPERIMENTS WITH BARIUM

It has proven very difficult to administer barium in doses sufficient to affect tetany without the production of very marked toxic effects, and our experiments are not, so far, very successful with this substance.

1127. February 24. Dog, weight 5.6 kg. Parathyroidectomy.

February 27. 11.25 a.m.—Distinct tetany. Respiration 84. Muscu-

February 27. 11.25 a.m.—Distinct tetany. Respiration 84. Muscular twitchings over whole body. KC-below 0.1; KO-0.5; AC-0.1; AO-0.5. 11.40— $\frac{M}{8}$ barium chloride run into jugular. After 2 cc. there was very marked increase in the severity of the tetany. 11.44 a.m.—3.5 cc. had been run in. Dog went into an epileptiform spasm and the respiration stopped in the expiratory position. Vocal cords apparently in a spasm, closing up the larynx. Tracheotomy was immediately performed and artificial respiration given but without success. On opening the thorax, the heart was found much dilated and the organs congested.

1139. February 25. Dog, weight 10.4 kg. Complete thyro-parathyroidectomy.

February 26. 10 p.m.—Found in violent tetany. KC-below 0.1; KO-not found; AC-below 0.1; AO-below 0.1. Respiration 240. Pulse uncountable. This dog was in most extreme tetany. 10.15 p.m.—About 8 cc. $\frac{M}{8}$ barium chloride was run into jugular. The dog suddenly fell into violent spasm of respiratory muscles, such that the chest became narrowed and inspiration seemed impossible. Artificial respiration was without effect. Tracheotomy was quickly done and further

artificial respiration, without effect. When the larynx was inspected from below it was found to be rigidly closed. Nothing could pass through the vocal cords, and even when forced apart they returned together. The muscles were still found to be twitching in the neighborhood. The heart was greatly distended. Apparently this type of laryngospasm occurs in frequent instances in which, without any injection, the dog is found to pass into an epileptiform seizure. It is probably the cause of death, which often occurs in that condition.

1156. March 22. Normal dog, weight 5.3 kg. KC-0.1; KO-tetanus at 2.8; AC-0.7; AO-1.6. At 2.55 p.m.— $\frac{M}{20}$ barium chloride run into the jugular. There was some difficulty about making the solution run into the vein and probably a little leaked. The level was lowered from 15 to 20 and the dog received about 3 cc. 3 p.m.—KC-less than 0.1; KO-tetanus at about 2.0; AC-0.4; AO-1.6. At 3.03 p.m.—2 cc. more were run into the vein. Dog suddenly went into opisthotonos. Very forcible respiration and died immediately with spasms in all the muscles. Just after his death; KC-0.1; KO-4.0; AC-0.6; AO-3.0. There was distinct twitchings of all the muscles with fibrillary tremors of the tongue. Most violent peristalsis of the intestines appeared and they discharged a quantity of watery material. The trachea was opened but the vocal cords stood quite apart. The heart stopped beating effectively, but when the chest was opened ten minutes after death, it was found to be giving slight twitchings. These twitchings are still distinct in the muscles of the abdomen and of the extremities and strong enough to move the feet.

1157. March 22. Dog, weight 6.2 kg. KC-less than 0.1; KO-tetanus at 2.0; AC-0.3; AO-1.0. At 3.25 p.m.—2.5 cc. $\frac{M}{20}$ barium chloride run into jugular. Dog licks his lips. 3.40 p.m.—Pulse 78, irregular. 3.45 p.m.—No special symptoms have appeared. KC-0.1; KO-tetanus at 1.8; AC-0.1; AO-0.7. 3.55 p.m.—1.25 cc. run into vein. Dog licks his lips. Marked salivation. Fibrillary twitching of tongue. Pulse 44, respiration about 12, long drawn. Seems well enough. 4 p.m. KC-0.1; tetanus at 1.4; KO-2.7; AC-0.4; AO-1.8. At 4.12 p.m.—1 cc. run in. In all 4.75 cc. of $\frac{M}{20}$ barium chloride had been given. Extreme salivation with wide dilatation of the pupils. Tremors of the tongue. KC-0.1; KO-2.0; AC-1.0-0.8; AO-tetanus at 2.2; AO-2.8. At 4.20 p.m.—Vomited thick mucus and bile. 4.30 p.m.—Walks with utmost stiffness—wide-spread legs. Defecated large amount of fluid material. No distinct twitching.

From these experiments it is evident that it would be impossible to effect a cure of tetany with the barium salts, because even though large doses of barium might be conceived of as having a dulling effect upon the motor nerves, the extreme toxicity would result in the death of the animal long before any such dose could be given; and in the doses which are tolerable, no such effect upon the motor excitability may be produced. It seems, therefore, not worth while in this connection to make any further experiments with barium.

EXPERIMENTS WITH POTASSIUM

Reference may be made to the experiments (108, 808, 1908) described in our previous paper.

1123. January 18. Dog, weight 5 kg. Complete thyro-parathyroidectomy.

January 19. Is well.

January 20. 10 a.m.—Severe tetany. Dog lying on side, breathing rapidly. Pulse uncountable. 10.30—Respiration 28, pulse 132. Tetanic contractions of all of muscles. 10.36 to 10.59 a.m.—35 cc. $\frac{M}{8}$ potassium acetate run slowly into jugular. During this time the tetany persisted, and toward the end became more severe, resulting in violent spasm with opisthotonos. 11.15 a.m.—Dog lying quiet but stiff, with occasional twitchings. KC-0.1; KO-1.5; AC-0.1; AO-0.3. 12.15 p.m.—Distinct tetany in all muscles. Violent tongue tremors. KC-less than 0.1; KO-1.2; AC-0.2; AO-1.1. 1.15 p.m.—Still has tetany. 4.45 p.m.—Pulse 120, respiration 40. Slight twitching and tremor of tongue. Much depressed. KC-less than 0.1; KO-1.4; AC-0.6; AO-1.0. 10.20 p.m.—Marked tremors of tongue. Shivering. No distinct tetany. 10.25 p.m.—KC-0.05; KO-1.8; AC-0.2; AO-0.8.

January 21. 11 a.m.—Mild tetany. 2.50 p.m.—Marked tremor of tongue with twitching of all muscles. Risus sardonius. KC-0.01; KO-0.01; AO-0.01; AC-0.01. 3-3.20 p.m.—50 cc. $\frac{M}{8}$ potassium acetate run into jugular. During this time there was a violent attack of opisthotonos, clenching of jaws and cessation of respiration. Later dog relaxed somewhat. There were snapping movements of the jaws and finally, the general tetany became much more severe than before the injection. Respiration 42, pulse 162. Response to stimuli was so active that it could not be measured with milliammeter. AO only was 0.1.

5.25 p.m.—Still severe tetany. Still impossible to record the measurements of KC and AC. KO-0.2; AO-0.01.

January 23. Dog still alive. Is sick but with no tetany. Dog became apathetic. Lived until January 28.

1146. March 2. Normal dog, weight 10.7 kg. 2.40 p.m.—KC-0.4; KO-tetanus at 10.0; AC-1.8; AO-7.0. Pulse 180, respiration 24. From 2.55 to 3.04—50 cc. $\frac{M}{8}$ potassium chloride run into jugular. After that: KC-0.4; KO-tetanus at 8.0; AC-2.0; AO-15.0. Pulse 160, respiration 24.

March 5. 7.30 p.m.—KC-0.7; KO-tetanus at 9.0; AC-3.0; AO-7.2.

It is pretty clear from these experiments that potassium has no effect in curing the tetany nor in diminishing the electrical excitability of the nerves. If anything, it seems rather to increase the excitability.

EXPERIMENTS WITH SODIUM SALTS

In a recent paper by Joseph and Meltzer⁴, there is made the statement that it is possible to cure the symptoms of tetany by the injection of a hypertonic solution of sodium chloride just as well as with injections of calcium salts. For this purpose they have found it necessary to inject 15-20 cc. of a molecular solution of sodium per kilogram weight of dog. We had performed this experiment once or twice some time ago with the following results:

7009. May 18, 1909. Small dog; two parathyroids and one whole thyroid lobe with parathyroids extirpated.

May 20. Dog found with twitchings of tongue but none elsewhere.

May 21. 9 a.m.—Dog found in most violent tetany. Extreme tachypnea. 10.05 a.m.—30 cc. 10 per cent sodium chloride introduced intravenously. This solution is not quite twice molecular. Unfortunately, the dog was not weighed, so that the relation to weight is not evident; but from the description in the notes, the dog probably weighed about 5-6 kg. At 10.10 a.m.—Still rigid. Respiration slower, 104 to the minute. 10.20 a.m.—Dog very quiet. Still rigid. Respiration 36 to the minute. Can walk about. Is exhausted and weak but remarkably improved. Slight twitchings are to be observed here and there.

⁴ Joseph and Meltzer. Jour. Pharmacol. and Exp. Ther. 1911. vol. II, p. 361.

Drinks very greedily. 10.30 a.m.—Dog is apparently all right. Sits up and runs about. 10.45 a.m.—Dog urinated abundantly. Drinks a great deal. 12.30 p.m.—Dog has been playing about. One would have scarcely any suspicion of tetany now. There are slight twitchings of some muscles. About 4 o'clock again began to have tetany which continued till 6 p.m. when he was given, by stomach tube, 7 grams of sodium chloride. Dog collapsed. Tetany stopped and I thought him dead. Artificial respiration was given. After awhile he breathed spontaneously and lay quietly on the table. Heart beat violently. 10.30 p.m.—Dog was breathing quietly. Hardly any suspicion of tetany.

May 22. 4 p.m.—Dog found twitching, but not very violently.

May 23. Dog pretty well but had moderate tetany. No tachypnea. No violent tremors. Given 5 drams sodium chloride in 200 cc. water.

May 24. Was stiff and threw himself about. Given two doses of 5 grams of sodium chloride but vomited both. Half an hour later no tetany. There are no further notes on this case.

7309. May 20, 1909. Dog, weight about 5.6 kg. Thyro-parathyroidectomy.

May 22. Tetany violent at 6 p.m. Give 7 grams salt by stomach tube with 200 cc. of water. At 12.00 Midnight, violent tetany. 1 a.m.—Extreme tetany, tachypnea, etc. High temperature. Given at 1.10 a.m.—40 cc. 10 per cent sodium chloride intravenously. 1.20 a.m.—Respiration still rapid. Lay rigid. No particular improvement. 1.25 a.m.—Respiration much quieter, only 40. Still rigid. 1.40 a.m.—Still twitchings in muscles but can walk about. Drinks greedily. Is stiff and unsteady but respiration is perfectly quiet and apparently he can be safely left for the night. Drinks a great deal and is still thirsty.

May 23. Dog found again in tetany which may have lasted all night but is not very violent. 11.45 a.m.—Respiration rapid and labored. Given about 5 grams of salt by stomach tube some of which he vomited. There are no further notes on this dog.

These two rather incomplete experiments further support the statement by Joseph and Meltzer, although, as it is seen, the influence on the tetany is a very ephemeral one. To these may be added another experiment made some time before.

809. January 13, 1909. Dog, weight 12 kg. 12.20—Bled 100 cc. 12.28 p.m.—2 m. sol. sodium chloride run into jugular vein. 12.35 p.m.—30 cc. have run in. Vomited at 12.36. Dog was catheterized. By

1.20 p.m.—100 cc. had run into the vein. Urine began to flow very pale and clear. At 1.46 p.m.—150 cc. had run in. Breathing very deep. Some twitchings in the muscles. Pulse full and strong, 192. Long sighing breaths. The urine moderated in amount and very pale. Respiration 12 to the minute. By 2.04 p.m.—200 cc. had been run in. Dog very restless. No convulsions. 2.18 p.m.—Twitchings about the neck quite distinct. Pulse 180. By 2.22—300 cc. had been run in. Distinct twitchings over whole body. 2.25 p.m.—Twitchings marked in all the muscles and shaking the dog's body. By 2.35 p.m.—350 cc. had been run in. Dog is quite rigid. Was bled to death at this point.

This last experiment, while showing the diuretic effect of the injection of salt solution, is pushed to such an extreme as to be no longer analogous to the other experiments. Apparently, it shows the effect of great disturbance of the osmotic index of the fluids of the body upon the muscles and possibly a specific action of NaCl. These experiments we have repeated recently with a view to the study of electrical excitability after the injection of the salt, as follows:

111. January 12, 1911. Dog, weight 6 kg. Complete thyro-parathyroidectomy.

January 15. Dog found stretched out stiff and twitching. Quite helpless. Respiration jerky. Tongue bitten. No tachypnea. 1.10 p.m. Is stiff and twitching though by no means at the maximum violence. Pulse 96, respiration 36. Heart beat very violent so as to appear in the abdomen. Continuous twitching in head. 1.15 to 1.40 p.m.—40 cc. of $\frac{M}{I}$ sodium chloride injected into jugular. During this time tetany seemed quite unchanged. Pulse 150-160. Breathing rapid, 32. 1.43 p.m.—Still helpless and stiff. Twitching marked over shoulders. Can walk, but very stiffly. 2.30 p.m.—Condition about same as before injection. Twitching but not violent convulsions. 6 p.m.—In exactly the same condition. Some twitching. Much apathy. Some stiffness and general helplessness.

January 16. Noon—Dull and apathetic. No twitchings. Died two days later of pneumonia.

118. January 16. Dog, weight 4.0 kg. Total thyro-parathyroidectomy.

January 17. No definite twitching.

January 19. Distinct tetanic twitching which are constant and fairly strong though not extremely so. 12.55—Pulse 132, respiration 18. Twitching marked. From 12.56 to 1.12 p.m.—80 cc. $\frac{M}{I}$ sodium chloride injected into jugular. After this the tremors were not so distinct but were still present in the tongue, etc. Respiration, 128, pulse 132. 3.30 p.m.—Distinct mild tetany. Pulse 124. Drinks a great deal of water and looks depressed.

January 20. Seems all right.

January 21. 11 a.m.—Found in severe tetany. 2.35 p.m.—Pulse 126, respiration 42. Tongue tremor. Slight twitchings of body. KC-0.1; KO-0.4; AC-0.01; AO-0.3.

January 22. 11.55 a.m.—Dog in marked tetany. Labored respiration, 68. Pulse, 168. Muscles twitching everywhere. KC- so low as not to be recorded, possibly 0.01; KO-0.3; AC-0.01; AO-0.01. From 12.12 to 12.25—80 cc. $\frac{M}{I}$ sodium chloride run into the vein. At first respiration 108. Twitchings strong. Dog rigid. This condition was maintained. At 12.40—KC-0.05; KO-0.4; AC-0.06; AO-0.2. Violent tetany. The whole body in convulsions. Respiration 78. Pulse 162. Abundant urination. Tetany of maximum violence. 12.55—Most violent attack of tetany. 1.15 p.m.—Tetany of extreme violence. 6 p.m.—Dog dead.

1116. January 17. Dog, weight 8.3 kg. Total thyro-parathyroidectomy. Well until January 22. 11.50 a.m.—Found with severe tetany. Had violent attack and then relaxed completely. KC-0.1; KO-2.1; AC-0.8; AO-1.0. This measurement was made while dog was perfectly relaxed, no tetany but with fibrillary tremors of tongue. 11.55 a.m.—Dull and apathetic. Respiration labored. 12.20—Distinct tetany. Grunting respiration. 12.45—Marked general tetany. Respiration 24, very labored. Impossible to count the pulse. KC-0.08; KO-0.2; AC-0.4; AO-0.4. It is to be observed that the electrical excitability is now very high as compared with 11.50, 1 hour ago, when the dog had had a violent attack and was in complete relaxation. Tetany is now present and undoubtedly the nerves have recovered from the exhaustion of an hour ago. Nothing was done to the dog. Exposure of the vein throws dog into opisthotonos with laryngospasm, complete rigidity and cyanosis. 12.55—Again perfectly relaxed. No tetany. Respiration 24, pulse 120. KC-0.1; KO-2.4; AC-1.1; AO-0.8. 1.40 p.m.—Another laryngospasm attack. Barks with metallic sound. Violent twitchings. 1.50 p.m.—Another attack of laryngospasm. KC-0.2; KO-0.4; AC—could not be determined;

AO-1.2. 1.55 p.m.— $\frac{3}{8}$ sodium chloride started into jugular. Between 1.55 and 2.08—166 cc. of this salt solution run into vein. Fibrillary tremors of tongue intense. Twitchings over shoulders. Respiration 16. Expiration very difficult. Pulse 120. 2.15 p.m.—KC-0.09; KO-0.4. AC-0.08; AO-0.5. 5.15 p.m.—Dog still in marked tetany. Evidently dog not much improved. Not so much as by the hypertonic solution. Respiration labored. 5.25 p.m.—KC-0.05; KO-0.7; AC-0.2; AO-1.2. Dog is rigid with much twitching. 5.33 to 5.42—166 cc. $\frac{3}{8}$ sodium chloride run into jugular. Respiration still grunting. Tetany quite marked. No special change. The effect is in sharp contrast to that in dogs that were bled and then infused with $\frac{3}{8}$ sodium chloride. Compare protocol in No. 1119. 5.50 p.m.—Respiration 42, labored. Pulse 120. Walks stiffly. Urinates abundantly. KC-0.1; KO-2.6; AO-0.6; AC-0.6. 6.50 p.m.—Still slight tetany. Respiration grunting. January 23. Dog seems pretty well. Died January 25 in tetany.

1119. January 18. Dog, weight 3.5 kg. Total thyro-parathyroid-ectomy.

January 20. 11 p.m.—Dog in violent tetany. Universal twitchings. KC-contractions with no appreciable current; KO-not determined; AC-0.2; AO-0.9. 11.15 p.m.—Bled 200 cc. from femoral. 200 cc. $\frac{3}{8}$ sodium chloride introduced into jugular. 11.27—Dog lies quiet. Tremors of tongue present. KC-0.6; KO-1.3; AC-1.7; AO-1.7. 11.55—Quiet. No twitching whatsoever. 12.10 a.m.—65 cc. bled from the femoral and 140 cc. of his own defibrinated blood run quickly into jugular. Fibrillary tremors of tongue became more marked. Twitching appeared in shoulder. KC-0.4; KO-1.5; AC-1.6; AO-1.6. 12.25 a.m.—Twitching in muscles most distinct. 12.33—Pulse 145, respiration 28. There is a general thrill in the muscle with isolated twitching. No violent tetany. There was really a striking change in the general character of the muscular condition between the time when the perfectly lax animal lay quiet after the first bleeding and the time after the injection of the defibrinated blood,—a more definite change than had been seen in any previous experiment of this kind.

January 22. 6 p.m.—Dog seemed practically normal.

January 23. Dog in distinct tetany. Infection occurred in wound of leg. KC-0.05; KO-1.8; AC-0.6; AO-1.0. 1.30 p.m.— $\frac{1}{10}$ normal sodium chloride run slowly into jugular. Twitchings general. Breathing labored, 48. Violent fibrillary tremors of tongue. Jerking of head. 1.30 to 1.40 p.m.—70 cc. were run in. Tetany is worse if anything. KC-0.05; KO-1.7; AC-0.1; AO-1.0. 1.45—Tetany quite violent. 2.45—

Dog much better. Imperceptible twitching. KC-0.05; KO-1.0; AC-0.1; AO-0.5. Dog did not develop tetany again but became apathetic and died on January 26.

1122. January 18. Dog, weight 4.7 kg. Complete thyro-parathyroidectomy.

January 20. 12.30 p.m.—Violent tetany with extreme tongue tremor. No tachypnea. KC-0.2; KO-0.7; AC-0.2; AO-0.4. From 12.35 to 12.50—94 cc. $\frac{M}{1}$ sodium chloride solution run into jugular. 12.50 p.m.—KC-0.2; KO-1.9; AC-1.6; AO-0.5. Twitchings became slighter. Tongue tremor still violent. No longer any twitchings in head. Dog stupid but can walk about. 1.05 p.m.—Is distinctly better. 4.30 p.m.—No tetany. Seems depressed. Pulse 100, respiration 26. KC-0.2; KO-1.6; AC-0.9; AO-0.4. 9.40 p.m.—Distinct general twitching. Fibrillary tremors of tongue present but dog seems pretty well. No tachypnea 9.45 p.m.—KC-0.2; KO-1.2; AC-0.4; AO-0.6. Dog found dead two days later.

1136. February 25. Dog, weight 11 kg. Total thyro-parathyroidectomy.

February 26. 11.30—Found in severe tetany. KC-0.01; KO-0.4; AC-0.01; AO-0.2. 11.45 $\frac{M}{1}$ sodium chloride run into jugular. Most violent tetany with tachypnea and general twitching. Pulse not obtainable. Between 11.45 and 12.00—200 cc. solution was run in. Up to 11.45 was still showing violent tetany. At 11.58 was much quieter. Twitching still marked with occasional tachypnea. Pulse 174. KC-less than 0.1; KO-not recognized on account of tetanus; AC-0.2; AO-0.2. At 12.00—Dog pretty quiet. Slight tachypnea, sometimes marked twitching. At 12.10 lies quietly with only occasional twitching of leg. Fibrillary tremors still in tongue and general slight twitching under skin. Urinary bladder distended. 12.25—Dog is quiet. Occasional twitching. KC-0.01; KO-no determination possible—tetanus; AC-0.2; AO-0.4. 12.40 p.m.—Dog quiet. Urinary bladder much distended. 2. p.m.—Dog urinated a great deal. Quiet. KC-0.05; KC-0.05; KO-not obtained—tetanus at 3.0; AC-0.3; AO-0.6. 3.45 p.m.—Distinct tetany again. Occasional tachypnea. 4.25 p.m.—In violent tetany. Tachypnea, fever and general rigidity. Respiration 240. KC-less than 0.1; KO-1.0; AC-0.2; AO-0.6. From 4.30 to 4.57—220 cc. $\frac{M}{1}$ sodium chloride run into the jugular. During this the dog became quieter, and at 4.50, although the dog was breathing rapidly the pulse had sunken to 140 and twitching had stopped. There is still a tremor and thrill of the muscles and fibrillary tremors of tongue. 5.00 p.m.—Breathing

labored, 50 to the minute. Twitchings practically gone. KC-less than 0.1; KO-tetanus at 0.8; AC-0.3; AO-0.4. Dog is stiff and awkward. 6.52 p.m.—After excreting a great deal of urine dog seems well. Walks about and takes interest in everything. Got up on hind legs to investigate cupboard. 10 p.m.—Dog seems quite well. Twitching, if any, observed with difficulty. Respiration a little labored.

February 27. 9 a.m.—No tetany but dog is depressed. 5 p.m.—No change in condition.

February 28. 9 p.m.—Dog has slight but distinct tetany. At 5 p.m. tetany is severe. Dog bled from the carotid, after which he lies quietly and seems much improved. Later bled to death.

The effect of the salt solution was studied further in a normal animal, as follows:

1152. March 7. Dog, weight 8.06 kg. KC-less than 0.1; KO-tetanus at 2.2; AC-0.4; AO-1.2. From 4.15 to 4.42—172 cc. $\frac{M}{1}$ sodium chloride run into jugular vein (i.e., 20 cc. per kg.). Slight fibrillary tremors of tongue. 4.45 p.m.—KC-less than 0.1; KO-not determined; AC-1.4; AO-4 to 5.5. Urinated a great deal. 5.15 p.m.—Drinks a great deal of water. 5.25 p.m. KC-1 to 1.2; KO-tetanus at 6.0; AC-3.6; AO-8.0.

March 8. 2.40—KC-0.8 to 0.2; KO-tetanus at 4.5 to 5.0; AC-0.8; AO-9.0.

1144. Dog, weight 3.2 kg. normal.

March 8. 10 a.m.—KC-0.3; KO-tetanus at 3.0; AC-1.5; AO-2.6. From 10.15 to 10.28—70 cc. $\frac{M}{1}$ sodium chloride run into vein. KC-0.2; KO-tetanus at 3.0; AC-2.6; AO-1.1. 10.35 a.m.—Dog passes abundant urine. Does not seem affected by the infusion. 11.15 a.m.—KC-0.4; KO-tetanus at 6.0; AC-1.2; AO-3.6. 12.35 p.m.—KC-0.7; KO-tetanus at 4.0; AC-1.0; AO-2.0.

It is quite evident from these experiments that the introduction of a large quantity of salt solution of concentration greater than that of the blood does indeed, as pointed out by Joseph and Meltzer, stop the symptoms of tetany in some cases even though, as is shown from these experiments, it does not greatly lower the excitability of the nerves. Indeed, in some cases it is hardly possible for our instruments to detect any lowering in the excitability of the nerve, but it seems propable that there must be at least sufficient lowering of the excitability to prevent the occurrence of spontaneous twitching. In all instances, the disappearance of the symptoms followed

immediately upon the *marked diuresis* produced by the injection of the salts. Even before the urine is evacuated from the bladder, its removal from the blood has the desired effect and we have come to regard this as a strong support for the idea that tetany is produced by some circulating toxic substance, so that in this instance we have the cure of the symptoms of tetany brought about by a mechanical process *differing sharply from the mode of cure of the symptoms effected by the introduction of calcium* and such substances which may be conceived of as merely rendering the nerves obtuse to the effects of the poison, although that poison is still circulating in the blood. Further support for this idea is found in the following experiment.

1126. February 24. Dog, weight 8 kg. Two parathyroids removed from one side, whole thyroid lobe removed from the other.

February 27. 12, noon—Dog found in severe tetany. KC-below 0.1; KO-1.2; AC-0.2; AO-below 2.0. At 12.30 p.m.—Molecular solution of glucose was injected into the jugular, 160 cc. being introduced between 12.30 and 12.59. During this time became somewhat more quiet, but at the end was still having distinct tetany. 1.02 p.m.—Put on floor. Urinates abundantly. 1.05 p.m.—Dog is running about looking normal. Twitchings have disappeared with the exception of those in the tongue where they are still distinct. 2.30 p.m.—KC-below 0.1; KO-2.2; AC-0.6; AO-2.8. Is having distinct tetany. Respiration 42. 4.45 p.m.—Dog seems quite well but there is still slight twitching.

From February 28 to March 4 dog showed only slight twitching but developed pneumonia and was found dead on March 4.

In this instance the beneficial effect apparently also depended upon diuresis.

The great contrast between amounts of salts necessary to stop tetany and the effective amount of NaCl is made clear in the following table:

Amount of Ca Sr and Mg. salts necessary for removing the muscular twitchings and the nervous hyperexcitability in tetany dogs

NUMBER OF EXPERIMENT	WEIGHT OF ANIMAL IN KG.	TOTAL OF CC. INJECTED	NUMBER PER KILO		
1115.....	7	45	6.4	$\frac{M}{8}$	Ca-lactate (intravenous)
1124.....	8.8	50	5.6	$\frac{M}{8}$	Ca-lactate (intravenous)
1124.....	8.8	40	4.5	$\frac{M}{8}$	Ca-lactate (intravenous)
1131.....	5.9	40	6.7	$\frac{M}{8}$	CaCl ₂ (intravenous)
1131.....	5.9	25	4.2	$\frac{M}{8}$	Ca-lactate (intravenous)
1131.....	5.9	38	6.6	$\frac{M}{8}$	CaCl ₂ (intravenous)
1131.....	5.9	20	3.4	$\frac{M}{8}$	Ca-lactate (intravenous)
1135.....	8	70	8.7	$\frac{M}{8}$	Ca-lactate (intramuscularly)
117.....	4.4	25	5.6	$\frac{M}{8}$	SrCl ₂ intravenous.
117.....	4.4	54	12.2	$\frac{M}{8}$	SrCl ₂ intravenous.
1113.....	7.1	31	4.3	$\frac{M}{8}$	SrCl ₂ intravenous.
1113.....	7.1	40	5.6	$\frac{M}{8}$	SrCl ₂ intravenous.
1132.....	6	50	8.3	$\frac{M}{8}$	SrCl ₂ intravenous.
1132.....	6	20	3.3	$\frac{M}{8}$	SrCl ₂ intravenous.
1132.....	6	50	8.3	$\frac{M}{8}$	SrCl ₂ intravenous.
1132.....	6	20	3.3	$\frac{M}{8}$	SrCl ₂ intravenous.
1117.....	9.9	40	4.4	$\frac{M}{8}$	MgSo ₄ intravenous.
1120.....	8.9	50	5.6	$\frac{M}{8}$	MgSo ₄ intravenous.
1120.....	8.9	50	5.6	$\frac{M}{8}$	MgSo ₄ intravenous.
1138.....	10.8	80	7.4	$\frac{M}{8}$	MgCl ₂ intravenous.
1138.....	10.8	24	2.2	$\frac{M}{8}$	MgCl ₂ intravenous.
1138.....	10.8	17	1.6	$\frac{M}{8}$	MgCl ₂ intravenous.
1138.....	10.8	1.1	1.1	$\frac{M}{8}$	MgCl ₂ intravenous.
			15-20	$\frac{M}{8}$	NaCl intravenous.

EXPERIMENTS WITH AMMONIUM SALTS

1137. February 25. Dog, weight 7.5 kg. Total thyro-parathyroid-ectomy.

February 26. Dog well until 6 p.m. when he was found in violent tetany. Tachypnea and twitching. 10.30 p.m.—Perfectly quiet Slight twitching under the skin. Twitching of the tongue. KC-less than 0.1; KO-0.9; AC-0.2; AO-0.7. Evidently the nervous excitability is high. Pulse 144, respiration 42. 10.55 to 11.25 p.m.—150 cc. $\frac{M}{8}$ ammonium chloride run into jugular. 10.58 p.m.—Respiration 22, deep and slow. Pulse 138. 11.35—Pulse 132, respiration deep, 36. KC-less than 0.1; KO-tetanus at 0.9; AC-0.1; AO-0.5. Dog walks

about and is quiet. No tetany brought on by the injection of this considerable amount of ammonium.

February 27. Seems all right. No signs of tetany.

February 28. 10 a.m.—Distinct tetany. Muscular twitchings. No tachypnea. At 11.10 a.m. still in moderate but distinct tetany. KC—below 0.1; KO-tetanus at 0.8; AC—below 0.1; AO-0.1. From 11.25 to 12.00—150 cc. $\frac{M}{8}$ ammonium chloride run into jugular. During this time dog was in quite marked tetany. Breathing 42 and very deep. Twitching remains about the same throughout. Does not seem to be affected at all by the injection of ammonium. At noon, the respiration is 30, very deep. Twitchings about the same. KC-less than 0.1; KO-0.8; tetanus at 1.4; AC-less than 0.1; AO-1.0. Dog walks about. At 4.45—Slight fibrillary twitchings of tongue. No muscular twitchings. Seems depressed. KC-less than 0.1; KO-1.1; AC-0.2; AO-1.2.

March 1. 11 a.m.—Slight tetany. March 2—Slight tetany.

March 3. Found dead. Autopsy revealed nothing. Dog probably died of tetany during the night.

1151. March 4. Normal dog, weight 6.4 kg.

March 5. KC-1.0; KO-tetanus at 1.4; AC-3.2; AO-3.6. 4.50—6.04—135 cc. $\frac{M}{8}$ ammonium chloride run into jugular. At 6.15—KC-1.3; KO-tetanus at 5.0; AC-4.2; AO-very high—10.4 to 11.0. Of course 135 cc. of fluid has been injected but it is clear that the motor excitability is not increased but rather seems to be distinctly depressed. Dog had no marked symptoms but vomited and licked his lips. Breathing rapidly.

March 6. 11.30 a.m.—KC-1.2; KO-tetanus at 3.6; AC-3.0; AO-4.0. From 11.45 to 11.52—74 cc. of $\frac{M}{4}$ ammonium chloride run into jugular. After 30 cc. dog began to vomit violently and have muscular jerking. Reflex excitability increased. At 12.00—KC-0.8; KO-tetanus at 5.0; AC-2.0; AO-2.6. Dog is comatose. Reflex increased. Tremors of tongue. 12.08—Marked salivation. Pupils dilated. Pulse 96, weak but regular. 12.15—KC-0.7; KO-tetanus at 4.0; AC-2.0; AO-9.0. Dog has regained consciousness. Looks very depressed. At 2.45—KC-0.6; KO-tetanus at 4.0 to 5.0. AC-1.8; AO-5.5.

March 8. 11.45 a.m.—KC-0.3; KO-tetanus at 3.2; AC-1.4; AO-3.2. With the elapse of two days the electrical excitability has apparently returned to normal. Dog was used then as a normal dog for experiment with strontium.

The experiments of Jacobson, Amer. Jour. Physiol. 1910, XXVI, 407, have concerned themselves with the estimation of

the amount of ammonium in the blood of dogs affected with tetany and have compared this with the amount of ammonia necessary to produce twitchings. They conclude that the amount in the latter case is the same as in the former and that therefore it seems possible to assume that the presence of an excess of ammonia may be important in producing tetany. The foregoing experiments, however, seem to show conclusively that the ammonia can hardly be responsible for the symptoms of tetany but is probably, rather, a result of the tetanic twitchings. This seems especially clear from the fact that injections of relatively enormous amounts of ammonia into dogs apparently upon the verge of tetany had rather a curative effect than otherwise.

Numerous experiments have been made with the aim of precipitating in the tissues the calcium salts by the injection of some substances such as oxalates or citrates, which have this effect. This work was principally carried out by Loeb and his students and also by several Italian investigators, whose work we have referred to in our previous paper. Stoeltzner, in his paper upon the pathogenesis of tetany, refuses to believe that the calcium can be precipitated extensively in such dilution. Nevertheless, the other investigators have succeeded in producing twitching of the muscles by the introduction of these salts. We had made one or two experiments in this direction, previously, but without any particular success nor have we been especially struck with the results in the experiments which we have done recently and which are as follows.

1145. March 3. Dog, weight 7 kg. 4.10 p.m.—KC-0.2; KO-tetanus at 3.6; AC-0.9; AO-5.0. Pulse 120, respiration 20. From 4.30 to 4.45—30 cc. $\frac{M}{8}$ sodium oxalate run into jugular. 4.40 p.m.—KC-0.2; KO-tetanus at 6.0; AC-1.6; AO-2.2. Some twitchings of the muscles. Fibrillary tremors of the tongue. Is quite relaxed and apathetic. 4.50 p.m.—KC-0.1; KO-tetanus at 2.8; AC-0.2; AO-1.2. Dog is distinctly dull and quiet in spite of the fact that the muscles twitch. Pulse very slow, 48. Respiration 15. General depression. Walks about stiffly just like tetany dog.

March 5. 6.25 p.m.—KC-0.1; KO-tetanus at 1.2; AC-0.1; AO-0.1. Shivers a great deal. Occasional fibrillary tremors of the tongue and lips.

March 6. 10 a.m.—Walking with stiff legs. Seems excitable. KC-0.1; KO-tetanus at 1.3; AC-0.3; AO-1.1. 10.20 a.m.—Pulse 136, respiration 20. 10.30–10.45 a.m.—50 cc. $\frac{M}{8}$ calcium chloride run into the jugular. After 5 cc. the pulse became very strong. At 10.33 when 30 cc. had been introduced: KC-0.1; KO-tetanus at 6.0; AC-0.3; AO-1.1. At 11 a.m.—After 50 cc. had been introduced for 15 minutes, KC-0.2; KO-8+; AC-1.2; AO-7.2. Dog has recovered from his depression.

EXPERIMENTS WITH XANTHIN

1144. February 28. Normal dog, weight 3.2 kg.

March 5. Dog in good condition. KC-0.1; KO-tetanus 3.6; AC-1.0; AO-1.4. Cocaine inserted. Dog seems somewhat quieter. 1.15 p.m.—KC-0.1; KO-tetanus at 3.6; AC-1.0; AO-1.4. 1.17 p.m.—Solution of Xanthin, 5 mg. per cc. was run into the vein. Dog shivers quite violently. Feels very hot. No fibrillary tremors of tongue. No twitchings. Temperature per rectum 40 C. At 1.45 p.m.—KC-0.1; KO-tetanus at 2.6; AC-1.2; AO-2.4. No twitchings whatever. Walks about perfectly well. Is happy. Normal looking. Passes a good deal of urine.

March 7. 3.50 p.m.—Dog is quite well. KC-0.1; KO-tetanus at 2.0; AC-0.3; AO-1.3.

The dog was used later for other experiments.

1150. March 4. Dog, weight 5.5 kg.

March 7. 2.45 p.m.—KC-0.2; KO-tetanus at 2.0; AC-0.2; AO-1.6. 2.53–3.05 p.m.—45 cc. of solution of Xanthin, 1 cc. of which equals 5 milligrams, that is, 225 milligrams were run into the jugular. No visible twitchings were produced. At 3.10 p.m.—KC-0.2; KO-tetanus at 4; AC-0.8; AO-1.5. 3.20 p.m.—Doubtful twitchings of tongue observed. Pulse 200, respiration 20. Dog looks depressed. 5.15—Excreted abundant urine and has drunk a good deal of water. Slight doubtful fibrillary twitching of tongue. KC-0.1; KO-tetanus at 1.8; AC-0.8; AO-1.2. The dog did not at any time develop any evidences of twitching.

The rate of infusion as well as the amount of Xanthin⁵ injected per kilo, were the same as in the experiments of Berkeley and Beebe.⁶

⁵ For these experiments we are indebted to Dr. Walter Jones of Johns Hopkins University, whom we wish to thank for a specimen of Xanthin which he prepared and the purity of which is attested by the following analysis: N- in Xanthin 36.84 per cent theoretically. N found in this specimen: 36.79 per cent; 36.98 per cent; 36.71 per cent.

⁶ Journal of Medical Research, 1909, xv, 171.

RÉSUMÉ

From all these experiments it appears that we have in the measurement of the electrical excitability of the motor nerves a most characteristic criterion of the existence of tetany. Even when the symptoms which are associated with muscular twitchings, fever, tachypnoea, etc., are absent, we may recognize their approach by the heightened excitability of these nerves. This affects all the measurements but the variation from normal in the KO and AO seem to be far greater than that of the KC and AC, and in acute tetany the figures representing the strength of current required to produce a contraction may in all four particulars be very small indeed—even below the 0.1 milliamperes which our meter would record. It is apparently within such low levels that the excitability becomes such as to allow of spontaneous twitchings, for by the aid of some salt injections, we are able to completely relieve the twitchings without lowering the excitability to a degree appreciable with our meter.

The use of the milliammeter, has, however, given us some clear light upon the mode of action of the various substances employed in the cure of tetany.

Thus, in the case of both normal dogs and those in tetany, we have found that injections of calcium, strontium or magnesium greatly lowers the excitability of the nerves and that therefore the figures representing the current required to produce contraction suddenly become very high. In addition to this relatively simple action on the part of the calcium and strontium, the magnesium seems to have a further action in abolishing the sensory reflexes and rendering the animal quite anaesthetic. This condition may be antagonized by calcium as pointed out by Meltzer, although the antagonistic action of the calcium upon that of the magnesium is not so striking in the case of the motor nerves, and in some instances seems to be merely superadded to it. At any rate, we know that each salt by itself will depress the excitability of the motor nerves although we have not analyzed sufficiently closely their mutual effect when injected together.

In such doses as promptly cure the symptoms of tetany, it

seems that these substances act essentially by dulling the excitability of the motor nerves and rendering them insusceptible to the influence of any circulating poisonous substance which may appear as the result of parathyroidectomy, or possibly by affecting some structure of the neuromuscular apparatus peripheral to the point of attack of the poison. Such poisons, then, give rise to no twitchings by way of the benumbed nerves although the animal still has perfectly facile control of his voluntary muscles.

In our previous paper we put forward the idea that in injecting calcium salts and thus producing a diminished excitability of the motor nerves, we were probably merely replacing the calcium necessary to maintain the normal excitability which had been altered by the disturbances produced by parathyroidectomy. This we based upon the observed diminution in the calcium content of the blood and brain and upon the observations made by many authors upon the effect of removing or precipitating calcium from the tissues—a procedure which regularly brought about such an increase in the excitability of the nerves, that spontaneous twitchings would appear. In this sense we regarded the calcium as a physiological constituent of the cell and thought of our injection of calcium as acting by the replacement of that calcium which had been lost and the consequent restoration of the physiological condition of the cell.

Now it seems that we must modify this view, although it is by no means disproven, since it appears that our efforts at the cure of tetany result in effects upon the nervous system which are so gross that we may not permit ourselves to draw from them conclusions as to what might result were we able to replace precisely any calcium which might have been lost from the nerve cell. With our injections of calcium, etc., in relatively large doses, it is more likely that we directly decrease, even to a point far below the normal, the excitability of the motor cells. It will remain, therefore, for further study to show whether it is in any way possible to restore the cells exactly to their normal condition and not to far overstep this point and benumb them.

Therapeutically, we still think calcium far superior to magnesium on account of the toxic effects of the latter. Barium is so

poisonous that no such results can be obtained with it, while strontium resembles more closely calcium in its effects. Injections even of calcium should not be made subcutaneously, however, because of the irritating and destructive local effects upon the tissues.

On the other hand, bleeding or the replacement of the blood in part by an indifferent salt solution will also relieve the symptoms of tetany although the hyperexcitability of the motor nerves is not greatly reduced in this way. This we have always had to interpret as being the effect of the removal of a circulating poison produced when the parathyroids are destroyed.

Quite analogous to this seems to be the results attained by the introductions of large quantities of $\frac{1}{4}$ sodium chloride solution of glucose and of other diuretics which seem to overcome the symptoms of tetany temporarily and very slowly by removing, through their diuretic action, quantities of fluid from the blood and tissues and with it the circulating poison. In this case it is not the action of the sodium ion but rather the somewhat mechanical effect of the diuretic which is beneficial.

There are thus two quite different ways in which the symptoms of tetany may be relieved by the action of inorganic substances. In the one case the increased tendency of the nerve cells to respond to stimuli is decreased by the action of calcium, magnesium, etc., so that the stimulating substance cannot so readily affect them, while in the other, the quiescence is apparently brought about by the removal by diuresis of the stimulating substance.

We would especially emphasize the fact that *in all our experiments we have never failed to cure the symptoms of tetany immediately with calcium, strontium or magnesium, whereas $\frac{1}{4}$ sodium chloride acts very slowly, and as can be seen from the protocols, in some instances, does not prevent death from tetany within a few hours.*

We have not been able to actually demonstrate the presence of a substance in the blood capable of causing these twitchings, although we have made many biological tests both of the whole blood and of various constituents such as ammonium salts, xanthin, etc., which might possibly be supposed to be present in excess in the body when metabolism is thus disturbed. Since

injection of the ammonium salts does not increase the electrical motor excitability, and even in a parathyroidectomised animal with heightened motor excitability, does not precipitate the tetany, we think that their presence in excess in tetany is the result of the violent muscular activities, etc., and not the cause of the tetany. Further, the convulsions which can be produced by large quantities of ammonium salts are quiet unlike the contractions of tetany and are very poorly antagonized by calcium salts.

Xanthin, even in quite large doses, does not produce twitchings nor even change the electrical excitability in any appreciable degree.

We have as yet no clear idea of the nature of the cause of tetany. The studies of metabolic changes after parathyroidectomy have given us no definite clue to the character of the disturbance, and the most promising field seems still to lie in the search for some actual toxic material in the circulating fluids.

THE RÔLE OF THE PORTAL CIRCULATION OF THE LIVER IN BILE FORMATION AND JAUNDICE

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From the classical experiments of Joh. Müller,¹ Kunde,² Moleschott³ on frogs, of Minkowski and Naunyn⁴ on geese, we know that bile pigments are almost exclusively formed within the liver. Virchow,⁵ however, was able to demonstrate the formation of an iron-free pigment in old blood extravasations and also at the site of subcutaneous injections of hemoglobin. He named this pigment hematoidin. Later researches furnished sufficient proof for the assumption that hematoidin is identical with bilirubin. Under normal conditions this second mode of formation of bile pigment may be neglected, as by far the greatest quantity of bile pigment results through the activity of the liver cells. For the bile acids an extrahepatic origin has not been demonstrated. Knowing that the typical bile constituents are exclusively derived from the activity of the liver cells, the question arises, What are the factors influencing this function of the liver? Since the liver is a gland supplied by nerves, one might think that possibly the production of bile is under the direct control of the nervous system. As a matter of fact this does not appear to be the case, as Pflüger⁶ and many later investigators could demonstrate that

¹ Joh. Müller: *Lehrb. d. Physiol.*, 1844, p. 131.

² Kunde: *De hepatitis ranarum extirpatione*. Berolini, 1850.

³ J. Moleschott: *Vierordt's Arch. f. physiol. Heilk.*, 1852, ii, 479.

⁴ Minkowski and Naunyn: *Arch. f. exp. Path. u. Pharm.*, 1886, xxi, 1.

⁵ Virchow: *Arch. f. Path. Anat.*, 1857, xii, 48.

⁶ Pflüger: *Arch. f. d. ges. Physiol.*, 1868, ii, 192.

section of the nerves leading to the liver did not abolish the formation of bile. It cannot be denied however, that the excretion and formation of bile is dependent at least *to some extent* upon nervous influences.

As far as the excretion of bile is concerned, the studies of Heidenhain and his pupils, and more recently of Doyon,⁷ have conclusively demonstrated that stimulation of the splanchnic nerves is followed by a constriction of the gall bladder, the bile ducts, and the sphincter muscle of the common duct in the duodenum. In this manner the flow of bile into the duodenum is temporarily increased. Doyon furthermore pointed out that certain drugs which are known to stimulate the nerve terminals contract the gall bladder (pilocarpin) or cause a relaxation of the bile passage (atropin).

That the nervous system, on the other hand, controls through a vasomotor mechanism the bile formation, had been shown by Heidenhain⁸ and Munk,⁹ and was later confirmed by many others. As experimental proof for this assumption these investigators called attention to the fact that continuous stimulation of the splanchnic nerves sometimes caused a decreased flow of bile from a cannula inserted into the gall bladder or common duct. This observation was explained by a *vasoconstriction* of the liver capillaries, which lead to a diminished perfusion of this organ.

Afanassiew¹⁰ pointed out, that section of the splanchnic nerves causes a *dilatation of the liver capillaries* associated with hyperaemia resulting in a better perfusion of the liver and causing bile pigments to appear in the urine and feces. This author explains the excretion of bile pigments in this condition by the dilatation of the liver capillaries causing a compression of the biliary capillaries, bringing about a stasis of bile, and consequently the bile constituents diffuse into the lymphatics and blood vessels and are finally excreted by the kidney.

⁷ Doyon: Arch. de physiol., 1893, p. 678 and 710; *ibid*, 1894, p. 30.

⁸ Heidenhain: Studien physiol. Inst. Breslau, 1868, iv, 226.

⁹ Munk: Arch. f. d. ges. Physiol., 1874, viii, 151.

¹⁰ Afanassiew: Arch. f. d. ges. Physiol., 1883, xxx, 385.

It is a common belief, that red blood corpuscles break down and lose their hemoglobin in the general circulation. The free hemoglobin is brought to the liver with the blood and gives rise to the formation of bile pigments. Stimulated by this assumption, a number of physiologists became interested in the question, *To what extent are the hepatic artery and the portal vein respectively responsible for the formation of bile?* The first experimental attempts in this direction were made by Oré,¹¹ who experimenting on dogs succeeded (as he thought) in eliminating the portal circulation by a gradual obliteration of the portal vein at the hilus of the liver. As in this case there was still some bile formed, he concluded that the blood of the hepatic artery was the source of bile formation.

Schiff¹² made extensive studies on the same subject and came to the following conclusions:

1. Ligation of the hepatic artery in cats does not prevent bile formation.
2. Ligation of the portal vein stops the bile secretion immediately and the animal dies within one or two hours.
3. Gradual obliteration of the portal vein (Oré) is not followed by a cessation of bile formation. A collateral circulation between the portal vein and the liver is established, and therefore this method does not yield conclusive results.

That the experiments of Oré and Schiff were not free from criticism was shown by Schiff¹³ himself in a fundamental research. He was able to demonstrate that the bile which is secreted into the duodenum is reabsorbed and carried by the portal circulation to the liver, to be reëxcreted into the intestines. Hence there exists a so-called circulation of the bile. In dogs with a biliary fistula Wertheimer¹⁴ more recently ligated the hepatic artery and found that the secretion of bile was not interrupted by such a procedure.

¹¹ Oré: Jour. de l'Anat et de la Physiol., 1864, i, 565; Compt. rend, 1856, ii, no. 9.

¹² M. Schiff: Schweiz. Zeitsch. f. Heilkunde, 1862.

¹³ M. Schiff: Giorn. di Scienze naturali ed Econ., Palermo, 1868, iv; Arch. f.d. ges. Physiol., 1870, iii, 598.

¹⁴ Wertheimer: Arch. de Physiol., 1892, iv, 577.

With these facts in mind we began a series of experiments the object of which was to study the influence of the portal circulation on the formation of bile. This work was made possible by the introduction of a method for establishing a permanent anastomosis between the portal vein and the vena cava inferior, thus leading the portal blood to the heart and preventing its passage through the liver. This method was first devised by Eck. In a previous communication¹⁵ we described a modification of this operation which simplifies the technique and decreases the mortality from 40 per cent to almost 0.

It was found that an Eck fistula combined with ligation of the portal vein at the hilus did not prevent the formation of bile. On the contrary, dogs thus operated upon remained healthy for many months, the operation obviously having no ill effects at all. In all these cases the gall bladder contained apparently normal bile, as shown by the usual tests for the various bile constituents. The stools did not have a fatty appearance, so that sufficient bile seemed to be excreted into the duodenum to bring about a complete absorption of fats. *Evidently in the absence of the portal circulation, the blood of the hepatic artery alone is sufficient for bile formation.*

The question then arose, What proportion of total bile formation is due to the portal circulation? This problem must of course be studied from a quantitative standpoint.

Two methods are at our disposal:

I. In animals with a biliary fistula the amount of bile formed is determined for a given time, after which an Eck fistula is made and the portal blood is led directly to the vena cava. Any influence of the portal circulation should now become evident by an estimation of the flow of bile for the same period of time as used before the establishment of the Eck fistula.

II. Ligation of the common duct in dogs always leads to a severe and fatal jaundice. On the assumption that the portal blood passing through the liver gives up some hemoglobin for the formation of bile pigments, this obstructive jaundice might possibly be modified in intensity by an Eck fistula.

¹⁵ Jour. Pharmacol. and Exp. Ther., 1910, i, 463.

We have chosen the second method and submit the results obtained in the following protocols. We hope to report in the near future further studies using the first method here mentioned. It is obvious that there are three possible ways of proving our point by means of the second method: The Eck fistula can be made (1) before, (2) simultaneously with, and (3) after the ligation of the common bile duct.

Before proceeding we may briefly refer to the symptoms occurring in dogs after the ligation of the common bile duct. To produce a permanent complete obstructive jaundice the common duct is dissected free near the duodenum and two strong ligatures are placed around it about 1 cm. apart, after which the part included between the two ligatures is resected in order to prevent the regeneration of the duct. Rosenberg¹⁶ has pointed out that one must take into consideration the possibility of an accessory duct which in case of obstruction of the main duct may still take up the function of the latter.

The succession of the symptoms is as follows: A few days (2 to 4) after the ligation of the bile duct, bile-pigments appear in the urine. A short time afterwards a jaundice is noticed in the mucous membranes and conjunctiva. The animal loses weight very rapidly and takes very little nourishment. The stools are clay colored. No urobilin is found in the urine. An anaemia develops and the number of red cells drops to one-half of the normal. Death occurs within three to four weeks. On autopsy it is noticed that the tissues are markedly jaundiced. Histological changes occur constantly in the liver. Small areas of necroses are found with regeneration of the biliary capillaries.^{17 18}

As a basis for our conclusions we have experimented upon six animals and submit the detailed reports of three of these experiments representing the three different types of procedure.

3810. Medium-sized female collie.

February 3. Dog had been starved for 24 hours.

¹⁶ Rosenberg: Arch. f. Anat. u. Physiol. (Physiol. Abt.), 1896, p. 191.

¹⁷ D. Gerhardt: Arch. exp. Path. u. Pharm., 1892, xxx, 1.

¹⁸ Joannoviez: Zeit. f. Kinderh., 1904, xxv, 25, (Pathol. Abt.).

Operation. Ether anaesthesia. Portal vein and vena cava sewn together side by side, following the same steps as in making an Eck fistula with the exception that no communication is made between the two vessels. Common bile duct cut between two ligatures. Wound closed. Dog recovers well.

February 11. Dog shows severe jaundice. Mucous membranes and skin yellow. Urine gives marked reaction for bile pigments.

Ether anaesthesia. Eck fistula completed and portal vein ligated near liver. Gall bladder much distended. Wound closed.

February 15. Dog takes some milk. Mucous membranes yellow. Dog shows marked improvement from this time on. Abdominal wound which had broken down healed and jaundice disappeared slowly.

March 1. Dog in fairly good condition. No jaundice. Is on a meat diet.

March 10. Dog lost some weight. No jaundice.

March 12. Eats very little. No jaundice. No bile pigments in urine.

March 14. Does not take food. Condition is getting worse.

March 15. Dog is very weak. Refuses food. No jaundice. No bile pigments nor bile acids in urine.

March 16. Found dead.

Autopsy: Dog is very emaciated. No subcutaneous fat left. Heart normal, contains some firm blood clots. Right lung normal. Left lung shows some areas of consolidation. Spleen normal. Adhesions between liver and diaphragm. Liver of dark brown color. Gall bladder contains a fluid of pale yellow color with a flocculent precipitate. Mucous membranes of stomach pale. Intestines normal. Pancreas pale. Right lobe of liver contains an abscess of considerable size involving the whole lobe and forming a cavity containing yellow pus (300-350 cc.). The wall of this cavity is 0.5 cm. thick and covered with yellow mucous.

Bile duct completely obstructed and separated from duodenum. Adrenals normal. Left kidney normal in size, capsule strips easily, on section pale. Right kidney shows a compression on superior pole, due to pressure from abscess. Capsule strips easily. Portal vein completely obstructed near liver hilus. No collateral circulation. Lymph glands in neighborhood of abscess enlarged. Peritoneum normal with exception of portions near abscess, where yellow patches are seen. No trace of jaundice in any tissue. Causes of death: Liver abscess, originating from upper end of infected wound. Microscopical examination

of section.¹⁹ Liver—Some acute congestion. Bile ducts appear perfectly normal and not distended. Liver cells in places contain small amount of bile pigment. No increase in connective tissue. Liver cells staining well. Practically no fat. Very little iron (Berlin blue stain).

3010. Small fox terrier. Weight 5 kg.

October 26. Starved.

October 28. 3 p.m. Ether anaesthesia. Incision in linea alba. Two ligatures are placed around common bile duct close to duodenum, part of duct included is resected. Gall bladder emptied. Strong silk ligature placed around portal vein near hilus but not tied. Eck fistula is made, after which ligature around portal vein is tied. Wound closed. Dog lost very little blood during operation. Comes out of ether very rapidly. Put into metabolism cage and is given water but no food.

October 29. Urine 400 cc. Test for bile pigments negative. Dog in fairly good condition but somewhat depressed. Pulse 90–100.

October 31. Dog seems perfectly normal. Passed 800 cc. of dark brown urine. Gmelin positive. Hammersten strongly positive. Liquid, clay colored stools. Pulse 120, regular, strong. No trace of jaundice.

November 1. Is put on meat and bone diet. Urine highly pigmented. Does not contain sugar nor albumin.

November 2. Slight yellowish tinge in conjunctiva. Urine contains much bile pigments.

November 5. Weight 6 kg. Skin wound broken down. Stools clay colored, no bilirubin nor urobilin. Jaundice completely cleared up. Animal bright and active.

November 8. Animal is quite well. Is still on meat diet. No trace of jaundice. Stools bulky, clay colored. Urine gives faint test for bile pigments.

November 14. No jaundice. Appetite poor, but animal is apparently in good condition.

November 15. Weight 5.25 kg. No bile pigments in urine.

November 23. Dog is emaciated. No trace of jaundice. Refuses meat, takes some bread.

November 24. Dog is in poor condition.

November 25. Found dead.

Autopsy. Is very emaciated. No adipose tissue left. No trace of jaundice. Heart and lungs normal. On upper end of wound, which

¹⁹ We are indebted to Dr. John H. King for the histological examination of the sections.

is badly infected, an abscess had developed which extends into peritoneal cavity in the region of the liver. Liver dark brown. Spleen, pancreas and kidney normal. No general peritonitis. Common bile duct well cut off from duodenum. No bile flows out of duct when pressure is put upon gall bladder. Bile ducts much distended. Ligature around portal vein secure. Eck fistula perfect, 8 mm. long. No collateral circulation. Sections taken from liver, adrenals, kidney and thyroid.

Microscopical examinations—Liver: Some bile pigments in liver cells and caniculi, otherwise normal. Kidney: practically normal. Small amount of parenchymatous degeneration of convoluted tubules.

3210. Male dog, weight 9 kg.

November 2. Dog had been starved for two days.

Operation: Ether anaesthesia. Eck fistula made and portal vein tied off near liver hilus. No hemorrhage. Dog recovers well, but refuses food for two days after operation.

November 4. Eats boiled beef. Looks perfectly well.

November 7. Wound healing.

November 8. Dog quite well. Turned out into yard and fed on boiled meat and bones.

November 14. Dog in very good condition. Wound healed.

December 3. Weight 9.35 kg. In excellent condition.

December 8. Hemoglobin 12.76 per cent. Red cells, 6,390,000. Leucocytes, 15,000.

December 16. Weight 8.7 kg. Dog had been starved for 24 hours.

Operation: Ether anaesthesia. Right rectus incision. A few adhesions loosened. Bile duct cut near duodenum between two ligatures. Wound closed. Dog recovers rapidly.

December 17. Takes some milk. Urine does not contain bile pigments.

December 18 to December 20. Faint trace of bile pigments in urine. Meat diet.

December 21. Weight 8.2 kg. Dog in excellent condition. Wound healed. No bile pigments in urine. No trace of jaundice.

February 6. Dog has been well up to this date. Has lost some weight, probably on account of poor care. No jaundice. No bile pigments in urine.

February 22. No jaundice. Emaciated.

February 23. Eats a considerable quantity of meat in the morning. Dies in the course of the afternoon. Cause of death: Ammonia intoxication.

Autopsy: Heart and lungs normal. Eck fistula 6 mm. Few adhesions between liver and stomach and intestines, but no collateral blood supply. Liver dark brown in color, congested. Bile duct not regenerated. Kidney normal. No trace of jaundice in tissues. Microscopical examination. Liver: Moderate amount of acute congestion, localized in lobules about the hepatic vein, in others about the portal vein. Occasional trace of bile pigment. Bile ducts appear normal, with slight increase in connective tissue around them. Liver cells contain a good deal of blood pigment.

NO. ANIMAL	HEMOGLOBIN	RED CELLS	LEUCOCYTES	REMARKS
	<i>per cent</i>			
3110.....	12.46	4,250,000	11,800	Eck fistula.
3210.....	12.76	6,390,000	15,000	Eck fistula + Obstruction of common bile duct.
3410.....	11.48	5,820,000	16,400	} Eck fistula.
3610.....	14.04	5,880,000	8,600	
4410.....	10.20	4,860,000	33,400	
Male.....	17.2	7,800,000	20,000	} Normal.
Female.....	13.4	5,150,000	16,400	
Normal Average.	12 to 14	6,500,000	10,000	

Methods used: Hemoglobin, Hemoglobinometer of Miescher-Fleischl; Cell count—Zeiss-Thoma.

This table illustrates the fact that there exists no marked variation in the hemoglobin content and the number of erythrocytes of the blood of animals with an Eck fistula and an obstruction of the common bile duct as compared with normal controls. The figures given in the above table are taken at random as typical from a large number of estimations.

The feces of these animals were tested a number of times for bile pigment and urobilin, but always with a negative result. This rules out the possibility of a compensatory excretion of bile pigment by the intestines.

From our experiments we are justified in drawing the following conclusions: In the absence of the portal circulation, ligation of the common bile duct in dogs did not produce jaundice. Should a mild jaundice develop during the first few days following the operation, it is probably due to a hemolytic action of the anaes-

thetic as a result of which more free hemoglobin is circulating with the blood and hence more bile pigments are formed. This overproduction of bile pigments is probably sufficient to explain this temporary jaundice.

In the six experiments five animals died of pneumonia, other infections (liver abscess) or meat intoxication; in no case, however, was jaundice present at death. One animal has survived and seems to be perfectly normal at the present time. As to the explanation of these facts, it seems obvious that the portal blood passing through the liver yields the material for the greater part of the total bile pigment formation; in other words, if the portal blood does not flow through the liver less bile is formed. How can this diminution in bile formation be explained? One might imagine that the portal blood contains a specific substance acting as a stimulant to the liver cells. This explanation is not very plausible since it has been shown that the blood from the hepatic artery alone is sufficient for the formation of bile.

Another explanation based on the following facts seems to us more rational. The liver is a very vascular and voluminous organ. Might it not be possible that red cells are broken down in this extensive network of blood capillaries? Before citing any evidence in favor of this new theory we shall sum up in short the generally accepted theory of bile formation, according to which red blood corpuscles disintegrate constantly in the general circulation. The liberated hemoglobin is removed by the liver and gives rise to bile pigments, the iron being deposited within the liver cells. Our own experiments are obviously not in accordance with this theory for the following reason: Removal of the portal blood, which is by far the greater amount of blood supplied to the liver in comparison with the arterial blood flowing to this organ, would lead to an accumulation of hemoglobin in the circulation, unless some other organ is able to take care of this free hemoglobin. This last possibility is not ruled out as such a function is often ascribed to the spleen. Joannovicz for instance claims that the spleen is the organ which takes up injured red cells. We have never been able to demonstrate any free hemoglobin in the serum of Eck fistula dogs.

The evidence in favor of our view that the liver is normally a blood destroying organ may now be considered. In this connection a series of investigations by Browicz²⁰ are of particular interest. This author found that the livers of normal dogs, which are killed during the process of digestion, reveal a remarkable histological picture. Within the liver cells are found complete red corpuscles and also hemoglobin crystals. Sometimes clumps of red cells are seen lying in well defined vacuoles. Furthermore, if a dog has received an intravenous injection of hemoglobin this substance is found in the cyto- and karyoplasm of the liver cells. According to Browicz such hemoglobin crystals are also observed in human livers from cases of chronic venous congestion and newborn children. Browicz claims to have demonstrated the presence of an intracellular network of capillaries which is in communication with the intercellular blood capillaries. The liver cells obtain their nourishment by means of these intracellular channels. Among other substances hemoglobin enters the nucleus, as is evident from the presence of hemoglobin crystals.

Herring and Simpson²¹ have been able to confirm these findings of Browicz. These authors injected the livers of various animals from the portal vein with carmin gelatine. Microscopical examination of such livers showed well defined intracellular capillaries. Red blood corpuscles in a process of disintegration and also hemoglobin crystals were frequently met with.

Herring²² in another publication gives a detailed description of the hemoglobin crystals found in the liver of dogs killed by chloroform. As the nuclear membrane in such cases is found intact, Herring believes that the crystals which he considers to be oxy-hemoglobin are formed *intra vitam*, because a sudden crystallization after the hardening of the cells is completed would lead to a rupture of the nuclear membrane. The importance of these histologic studies is evident in connection with the experimental work reported in this paper, as they furnish additional evidence

²⁰ Browicz: Arch. path. Anat. u. Allg. Path., 1902, clxviii, p. 1.

²¹ Herring and Simpson: Jour. of Physiol., 1906, xxxiv, Proc.

²² Herring: Jour. of Physiol., 1906, xxxiv, Proc.

for the following considerations: The liver in extrauterine life is actively engaged in the destruction of red cells. The erythrocytes are brought to the liver, enter the liver cells, disintegrate, the hemoglobin is changed into globulin, bile pigment and iron. The iron derived from the hemoglobin is deposited within the liver, whereas the bile pigments leave the cell by way of the intracellular bile capillaries, which are in communication with the intercellular system of bile ducts.

Kupffer²³ and Heinz²⁴ do not accept the existence of an intracellular capillary system, but claim that the endothelium of the liver capillaries 'has a phagocytic property for erythrocytes, allowing these cells to enter the liver cells. On this assumption our results can be explained just as well as with the view held by Browicz.

The liver has been shown by many investigators to form red cells during embryonic life (Howell²⁵). In recent years a number of investigators have discovered erythroblasts in the liver in certain diseases (pernicious anaemia), and are therefore inclined to adopt the view that this organ can assume a hematopoietic function after birth, if there is a demand for such an activity on the part of the body.^{26,27}

The liver of an adult mammalian animal (dog) is apparently therefore an organ which is actively engaged in the destruction and under certain conditions the reproduction of red cells.

Numerous authors have suggested that the spleen is concerned in the destruction of red blood corpuscles. The evidence in favor of this view is based chiefly on the findings of fragments of red cells within the cells of the spleen and on the comparatively high iron content of this organ. It is obvious that such proof is very deficient. On the other hand Joannovicz²⁸ has shown that after the introduction of hemolytic substances into animals (dog) the

²³ Kupffer: Arch. mikr. Anat., 1899, liv, 252.

²⁴ Heinz: ibid, 1901, lviii, 576.

²⁵ Howell: Jour. of Morph., 1891, iv, 58.

²⁶ Lobenhoffer: Beitr. path. Anat. u. allg. Path., 1908, xliii, 124.

²⁷ Meyer Erich: Münch. med. Woch. 1908, lv, 1161.

²⁸ Loc. cit.

spleen takes up the injured blood cells and destroys them completely. The liberated hemoglobin is carried to the liver and forms bile pigments in such amounts that jaundice is produced.

Removal of the spleen in obstructive jaundice has no influence on the course of the disease. It is therefore hardly possible that the spleen, if it takes any part at all in the etiology of jaundice, will occupy the same importance as the liver and its portal circulation.

CONCLUSIONS

1. The amount of bile formed by the liver depends on the blood flow through this organ.
2. The jaundice and fatal toxemia resulting from occlusion of the common bile duct can be avoided by an Eck fistula made at the time of the ligation of the duct.
3. In cases in which, owing to occlusion of the duct, jaundice has already developed, an Eck fistula causes the symptoms to disappear.
4. It is shown that the liver has a hemolytic function.

NOTE CONCERNING THE LAXATIVE PROPERTIES OF THE TRIBASIC SALTS OF PHENOLPHTHALIC ACID

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From the Pharmacological Laboratory of the Johns Hopkins University

Received for publication, May 1, 1911

Owing to its efficiency as a laxative, phenolphthalein at present occupies a position of great importance in modern therapeutics. It has attained great popularity with the profession and also with drug firms in general by whom it is exploited alone or in combination with other laxatives under various trade names as Phenolphthaline laxative, Probilin, Prunoids, Laxine, Laxaphen, Exurgine, Phenalein, Phenolax wafers, Thalosen, Laxothalen tablets, Veracolate, etc.

Its pharmacological properties and its efficiency as usually administered by mouth have tempted various workers to utilize it or one of its salts or substitution derivatives as a subcutaneous purgative. Fleig¹ has introduced into medical practice as a hypodermic purgative, a soluble derivative of phenolphthalein which he has called "sodophthalyl." Abel and Rowntree² and Rowntree³ have demonstrated the value of phenolphthalein and more particularly of phenoltetrachlorophthalein in this connection when administered subcutaneously in solution in olive oil. It is established beyond doubt that these preparations possess very valuable laxative properties when so administered, and also that no general undesirable concomitant action is produced elsewhere in the organism. It must be admitted, however, that the bulkiness of injection (20 cc. of the oil solution) stands in the way of

¹ Archives Internat. de Pharmacodyn. et de Thérapie, xviii, 327.

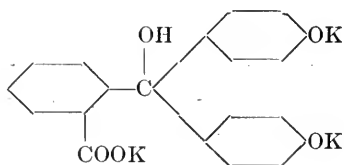
² This Journal, vol. i, p. 231

³ Jour. Amer. Med. Assoc., 1910, vol. liv, p. 344.

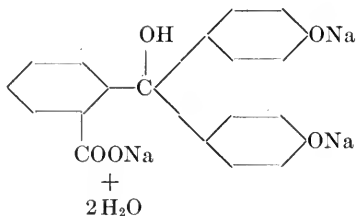
its general adoption, although it does not detract from its efficiency when a subcutaneous laxative is needed.

Through the kindness of Drs. Kober and Marshall of the Research Laboratory of the Roosevelt Hospital, New York, we have lately had the opportunity of investigating the pharmacological properties of potassium phenolphthalate and sodium phenolphthalate. These are the tribasic colorless salts of phenolphthalic acid which have been prepared for the first time by Kober and Marshall.

Potassium phenolphthalate is a light yellowish brown crystalline salt, which turns pink on being exposed to the air for a few hours.



Sodium phenolphthalate is a pinkish yellow crystalline salt, which rapidly becomes strongly pink and finally red on exposure to the air.



Both of these salts readily go into solution in water giving only a faint red color to the solution which has, however, an exceedingly strong alkaline reaction. It became necessary, therefore, to neutralize this solution prior to its subcutaneous administration.

The solution for injection of either of these salts is prepared as follows: To 1 or 2 gm. of the salt in a sterile glass bowl are added 1, 2, or 5 cc. of distilled sterile water, the amount added depending on the dilution desired. The salt readily dissolves, yield-

ing a clear pink or red solution which is strongly alkaline in reaction. To this is added dilute acetic acid⁴ (25 per cent) drop by drop; the solution effervesces and becomes deep red in color. The acid is added carefully until the red color entirely disappears, leaving a clear slightly yellow transparent solution which is neutral or even slightly acid to litmus. It is then immediately taken up in a sterile syringe and injected subcutaneously.

By this method a gram of either salt may be obtained in solution in as small a quantity as 1 cc. of fluid. In most of our experiments we prepared the solutions so that 2 gm. would be contained in 5 cc.

This solution must be injected immediately, for if allowed to stand it becomes gradually and progressively alkaline in reaction, so that within one hour it has again taken on a decided red color and a white precipitate has formed. It cannot be heated, for the colorless neutral solution is decomposed even by gentle warming, resulting in an intensely red solution which is strongly alkaline in reaction.

When a dilute solution is desired, water must be added prior to neutralization, or else a weaker solution of acetic acid employed. The addition of even a drop of water to the neutral solution causes a white precipitate to be thrown out, which is re-dissolved upon shaking, but if larger amount are added the precipitate is thrown out and is only dissolved in a great excess of water.

Experiments were made to see if these phenolphthalates possess purgative properties. The neutral solution above described was injected into dogs which were kept in separate metabolism cages and whose diet and water had been carefully controlled and the condition of whose stools was known. Only such dogs whose stools were well formed, were hard, dry and friable, were used. The skin was shaved in spots and the drug injected under aseptic conditions and with antiseptic precautions.

The data relating to these experiments are given in Table I.

⁴ Acetic acid must be used in this connection as the addition of any of the mineral acids gives a copious white precipitate immediately upon the neutral point being reached.

At first it appeared that no local irritant effect⁵ accompanied the subcutaneous administration of these preparations, but in about half of the experiments evidence of local irritant effects did appear, sometimes only as a slight indurated nodule appearing within a week or two after the injection, again as an infiltration involving a considerable area and appearing within two or three days. Undoubtedly these preparations⁶ are irritant locally.

These substances are of little or no value as subcutaneous purgatives and do not compare at all favorably with phenolphthalein itself or its tetrachlor derivative. Table II indicates the comparative values as purgatives of phenolphthalein, its tetrachlor derivative and di-sodium phenolphthalein, the data incorporated being obtained from the protocols of dogs, each of which under identical conditions received 0.2 gm. pro kilo of body weight of one or other of these substances.

TABLE I

DRUG AND DOSE	WEIGHT OF DOG IN KG.	DATE	PURGATION	DRUG IN STOOL	DRUG IN URINE	REMARKS
1 gm. potassium phenolphthalate	5.8	11-28				Marked infiltration occurred necessitating several incisions two weeks later.
		11-29	—	—	+	
		11-30	+	+	.	
		12-1	—	+ trace		
		12-2	—	—		
1 gm. potassium phenolphthalate	7	11-28				
		11-29	+	—		
		11-30	+	+	+	
		12-1	+	—	+	
		12-2	+ very slight	—		
1 gm. potassium phenolphthalate	7	11-30				Slight infiltration occurred at point of injection—a small hard nodule.
		12-1	no feces		+	
		12-2	+	+	+	
		12-3	—		+ trace	
		12-4	—		+	

⁵ At this date our results appeared most favorable and we so wrote to Kober and Marshall who unfortunately incorporated this information in their publication.

⁶ The addition of acetic acid for purposes of neutralization results in a hypertonic solution of sodium acetate which in itself may be somewhat irritating. Five cc. solution of sodium acetate of equivalent strength however, was injected under the skin in three dogs, but neither inflammation nor sterile infiltration appeared in any instance.

TABLE I (Continued)

DRUG AND DOSE	WEIGHT OF DOG IN KG.	DATE	PURGATION	DRUG IN STOOL	DRUG IN URINE	REMARKS
1 gm. sodium phenolphthalate	7	12-14				
		12-15	+	+	+	
		12-16	+	+	+	
		12-17	+ slight			
		12-18	-			
		12-19	-			
1.5 gm. sodium phenolphthalate	9.7	12-14				
		12-15	+ slight	+	+	
		12-16	-	+	+	
		12-17	-			
		12-18	no feces			
2 gms. sodium phenolphthalate	9.5	12-16				
		12-17	+?very slight	+		Considerable pain at time of injection.
		12-18	+ very slight	+	-	Slight local infiltration.
1.5 gm. sodium phenolphthalate	9.7	1- 7				
		1- 8	no feces		+	Pain at time of injection.
		1- 9	-	-	+	Neck somewhat infiltrated.
		1-10	+ slight	+	+	1-17-11.
		1-11	-	+ trace		
		1-12	-	+	+ trace	
		1-13	-	-	-	
1.6 gm. sodium phenolphthalate	7	1-9			+	
		1-10	+ slight	+	+	
		1-11	-	+	+	
		1-12	-	-	+	
		1-13	+ slight	+ trace	+ mere trace	
		1-14	-	-	-	
1.8 gm. sodium phenolphthalate	7	1-10				
		1-11	+ slight	+	+	
		1-12	-	+	+	
		1-13	+ ? slight	-		Some slight infiltration locally at point of injection.
		1-14	+ slight	-	+	
		1-15	-	-	+	
2 gms. sodium phenolphthalate	9.5	1-13				
		1-14	no feces		+	
		1-15	+ slight	+	+	
		1-16	+ slight	+	+	Some slight infiltration at point of injection.
		1-17	-	-	-	

TABLE II

DRUG	WEIGHT IN KG.	DATE	PURGATION	DRUG IN STOOL	DRUG IN URINE	REMARKS
Sodium phenol- phtalate 1.94 gm. made up to 5 cc.	9.7	2-7				Only on one day 2-13 was there any sugges- tion of a laxative effect.
		2-8	—	—	+	
		2-9	—	—	+	
		2-10	—	+	+	
		2-11	—	+	+	
		2-12	—	+		
		2-13	—	—	—	
Disodium salt of phenolphthalein 1.9 gm. in 60 cc.	9.5	2-7				For three days the stools were unformed and very soft and homo- geneous in consistence No local irritation.
		2-8	+ slight	+	+	
		2-9	+	+	+	
		2-10	+	+	+	
		2-11	+	+	+	
		2-12	+	+	+	
		2-13	+ slight	—	+ slight amount	
		2-14	+ slight	—	—	
Disodium salt of phenolphthalein 1.94 gm. in 90 cc.	9.7	2-18				Soft fluctuating swelling on the side 2-19. This partially disappeared in a few days. A defi- nite infiltration present in same area on March 1. Still a trace of drug in stools on March 1.
		2-19	no feces		+	
		2-20	+ slight	+	+	
		2-21	+	+	+	
		2-22	—	+	+	
		2-23	+	+	+	
		2-24	+	+		
		2-25	no feces		—	
Phenoltetrachlor- phthalein 1.48 gm. in 90 cc. olive oil	7.4	2-11				Trace of drug in urine but these were not catheter specimens. Stools were formed but soft in consistence and dark in color during most of this period.
		2-12	—	—		
		2-13	+	+	trace	
		2-14	+	+	trace	
		2-15	+ ? slight	+	—	
		2-16	+	+	—	
		2-17	+	+	—	
		2-18	—	+	—	
		2-19	+ slight	+ trace	—	
		2-20	—	—	—	
Phenoltetrachlor- phthalein 1.9 gm. in 120 cc. olive oil.	9.5	2-21				Stools fairly formed ex- cept on 2-25. Trace of drug in urine—not ca- theter sample.
		2-22	+ slight	+	trace	
		2-23	+	+	—	
		2-24	no feces		—	
		2-25	+ semi fluid	+		
		2-26	—	+	—	
		2-27	+	+	trace	
		2-28	+	+	trace	
		3-1	—	trace		

In an earlier communication Abel and Rowntree⁷ compared the relative purgative value of phenolphthalein and its tetrachlor derivative and demonstrated that the tetrachlor body exerted a more prolonged action when the two drugs were administered in olive oil in equal amounts pro kilo of body weight—the dose utilized being 23 mg. pro kilo. A study of Table II shows that this does not obtain when larger doses, 200 mg. pro kilo, are utilized, the tetrachlor derivative being administered in olive oil and the phenolphthalein as the disodium salt.

It was not our intention to publish these results except possibly in brief at a later date and only in relation to a further study of members of the phthalein family, but the results of our earlier observations prematurely alluded to in the recent publication by Kober and Marshall⁸ has made it incumbent upon us to report in full the actual results obtained in the pharmacological study of these new salts of phenolphthalein.

⁷ Loc. cit.

⁸ Jour. Amer. Chem. Soc., 1911, vol. xxxiii, p. 59.

STUDIES ON THE CIRCULATION IN MAN. IV. THE INFLUENCE OF OXYGEN INHALATION ON THE CIRCULATION IN A CASE OF CYANOSIS

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The relation of the blood gases to the circulation has excited much attention in recent years.¹ I have had the opportunity of measuring the blood flow in the hands of a man suffering from chronic bronchitis with marked emphysema and recurring cyanosis not associated with dyspnoea, and have studied the influence of inhalation of oxygen on the flow.²

The oxygen was administered in all the experiments except one by means of a mask. In this one experiment it was inhaled through a tube held in the mouth. In all cases the oxygen first passed from the cylinder to a good sized wash bottle through which it bubbled, the bottle being connected with the mask or

¹See especially Yandell Henderson: *American Journal of Physiology*, 1908, xxi, p. 126; 1909, xxiv, p. 66; 1910, xxv, p. 385; 1910, xxvi, p. 260; 1910, xxvii, p. 152, etc.; Hill and Flack: *Journal of Physiology*, 1910, xl, p. 347; Bayliss: *Journal of Physiology*, 1901, xxvi, p. xxxii; *Ergebnisse der Physiologie*, 1906, p. 345, etc. In these papers the literature is fully referred to.

²The method of measuring the flow was communicated to The American Physiological Society, December, 1910 (*American Journal of Physiology*, 1911, xxvii, p. xx). A detailed account of the method, constituting paper I of this series, with a summary of results obtained by it in a large number of experiments on normal persons and in clinical cases, has been sent to *Heart* (vol. iii, No. 1, 1911). A shorter summary was published in the *Cleveland Medical Journal*, May 1, 1911, p. 385. Paper II of the series "The effects of reflex vaso-motor excitation on the blood flow in the hands," has also been sent to *Heart* (loc cit.). Paper III, "The influence of forced breathing on the blood flow in the hands," appeared in the *American Journal of Physiology*, 1911, xxviii, p. 190.

tube, so that the subject breathed a mixture of air and oxygen. All the blood flow estimations were made with the subject in the sitting position, the hands hanging down. The observations on the case of cyanosis were mainly carried on in conjunction with experiments by Drs. J. J. R. Macleod and C. F. Hoover on the alveolar gas tensions and the influence exerted on them by oxygen. Since, however, these last observations are still incomplete and it is uncertain at what time a sufficient amount of material may be available to enable satisfactory conclusions to be drawn from them, I desire to publish now my observations on the blood flow, which yielded perfectly sharp and consistent results. My colleagues have kindly permitted me to include specimens of the observed gas tension measurements (see table, p. 496). The first observation, without any study of the effects of oxygen inhalation, was made on December 16, 1910. The protocol deserves to be quoted because it shows the amount of the flow in the patient after a period of rest and treatment in the hospital and thus affords a useful standard of comparison with flows measured under less favorable conditions.

S. G., tailor's presser. Age 44. Weight 140 pounds. Height 5 feet, 2 inches. Patient at Lakeside Hospital. He had occasionally presented himself at the dispensary for the past nine months suffering from chronic bronchitis with a high degree of emphysema. A certain degree of cyanosis was always to be observed although it was not equally great on different days. On one and the same day little if any difference could be detected at different times in the depth of the cyanosis. But from time to time he became more markedly cyanotic and the cyanosis was not associated with any change in the respiratory movements so far as could be made out. The respiration was of the "group" type. His hands felt warm and the veins were much dilated. The hands obviously contained at least the normal proportion of blood and probably more. To determine whether this was correlated with a normal onward flow of the blood or with such obstruction to the venous return as diminished the mass movement, the flow was measured at the request of Dr. C. H. Hoover, under whose care the patient was at the time. Only the right hand was examined on this occasion as the space for working was rather cramped.

3.17 p.m. Right hand put into bath at 30.2°. Room temperature 22.5.
Pulse (sitting) 74. Rectal temperature 37.2.

3.31. Right hand put into calorimeter A. Calorimeter B used
as a control to give the amount of heat lost by A to the
surroundings. 3050 cc. of water in each calorimeter.

TIME	A	TIME	B	NOTES
3.30	29.49			
3.33	29.50			
3.34	29.59			
3.35	29.70			
3.36	29.80	3.37	29.40	
3.38	30.00	3.39.30	29.37	
3.39	30.08	3.42	29.31	
3.40	30.16	3.45	29.26	
3.41	30.26	3.49	29.20	
3.43	30.42			
3.44	30.54	Hand taken out of calorimeter at 3.44. Room 23.3°		

Volume of right hand in calorimeter 438 cc.

Taking the temperature of the arterial blood as 36.7 (in a normal man it was shown to be 0.5° below rectal temperature), we get for the 11 minutes of the experiment a flow of 64.22 grammes of blood per minute, *i. e.*, 14.66 grammes per 100 cc. of hand per minute, with a mean calorimeter temperature of 30.02 and an average room temperature of 22.9. This flow instead of being less is considerably greater than that found in the normal persons examined under similar temperature conditions. Therefore, so far as the hand circulation can be taken as an index, the cyanosis is not dependent upon an abnormally small mass movement of the blood. Subsequent observations on the same patient confirmed this result. Although this was with one exception the greatest flow observed in his hand, he always showed a flow quite as great as the average normal person of his age, and indeed usually greater.

The next experiment shows the effect of oxygen inhalation.

S. G., January 20, 1911:

11.34 a.m. Hands put into bath at 30.0°. Room 18.6°. Mouth temperature 37.16°. Rectal temperature 37.6°. Pulse (sitting) 96.

11.47.15 Hands put into calorimeters. As usual, right into A, left into B.³ 3050 cc. of water in each.

TIME	A	B	NOTES	TIME	A	B	NOTES
11.46	29.23	29.04		12.16	30.47	30.34	
11.49	29.29	29.11		12.17	30.53	30.42	
11.50	29.35	29.17		12.18	30.60	30.50	
11.51	29.40	29.22		12.19	30.69	30.58	
11.52	29.47	29.30		12.20	30.76	30.64	
11.53	29.52	29.37		12.21	30.81	30.70	
11.54	29.58	29.42		12.22	30.88	30.78	
11.55	29.66	29.50	Room 19.4	12.23	30.92	30.81	
11.56	29.70	29.55		12.24	30.98	30.87	
11.57	29.75	29.61		12.25	31.05	30.94	At 12.25-45 mask taken off.
11.58	29.79	29.65		12.26	31.10	31.00	
11.59	29.85	29.71		12.27	31.16	31.05	
12.00	29.90	29.79	Room 19.4	12.28	31.22	31.11	
			Hands taken out and dried at 12.00	12.29	31.27	31.14	
			Replaced in calorimeters at 12.02	12.30	31.30	31.19	Room 19.4
				12.31	31.33	31.22	
12.04	29.90	29.80		12.32	31.39	31.28	
12.05	29.99	29.87		12.33	31.41	31.30	
12.06	30.03	29.91		12.34	31.46	31.34	
12.10	30.17	30.06		12.35	31.47	31.38	
12.11	30.20	30.09	Room 19.4	12.36	31.52	31.42	
12.12	30.24	30.12		12.37	31.54	31.45	Room 19.4, hands taken out at 12.37
12.12-30			Mask put on				
12.13-30			Oxygen started	12.47	31.38	31.29	
12.14	30.33	30.21		12.54	31.25	31.18	Room 18.7
12.15	30.39	30.27		12.57	31.20	31.11	

Volume of right hand in calorimeter 435 cc., of left hand 400 cc.

³ In the protocols it is to be understood that this was always the arrangement unless the contrary is stated.

For the first 11 minutes of the experiment before oxygen inhalation was begun, the flow was 37.01 grammes per minute or 8.51 grammes per 100 cc. of hand per minute for the right hand (with mean calorimeter temperature 29.60) and 39.18 grammes per minute or 9.79 grammes per 100 cc. of hand per minute for the left hand (with mean calorimeter temperature 29.45). For 11 minutes during inhalation of oxygen the corresponding flows were 49.87 grammes and 11.46 grammes for the right hand (with calorimeter temperature 30.69), and 49.17 grammes and 12.29 grammes for the left hand (with calorimeter temperature 30.58), an increase of 34.6 per cent for the right hand and 25.5 per cent for the left hand.

It is an interesting point, although too much stress must not be laid on a calculation of this kind, that the percentage increase is less in the left hand than in the right. For the original flow per 100 cc. of hand was greater in the left than in the right, a rather uncommon condition in a right-handed person and without doubt dependent upon a relative local vaso-dilatation of the left hand or a relative local vaso-constriction of the right. If the action of the oxygen is on the vaso-motor center or the peripheral vaso-motor mechanism, it might be expected that the increase in vaso-dilatation would be proportionately less in the previously dilated area than in the previously constricted area. An action of the oxygen which augmented the activity of the heart would, on the other hand, have no tendency to alter the proportion between the flows in the two hands. It is probable that by artificially establishing a difference in the flow in the two hands, as can be done in various ways, for example by immersing them in water at sufficiently different temperatures, or by taking advantage of spontaneous differences, conditions (or substances) which affect purely the heart's activity can be discriminated by this criterion from those which affect purely the vasomotor mechanism. The possibility of using this as a pharmacological method is being investigated.

If we take the flow for 7 minutes before inhalation of oxygen was begun as the base line from which the increase under oxygen inhalation is to be computed the percentage increase is much

greater than that calculated for the 11-minute period. For the 7 minutes the flow for the right hand was only 29.27 grammes per minute or 6.73 grammes per 100 cc. of hand per minute, and for the left hand 28.53 grammes per minute or 7.13 grammes per 100 cc. per minute. The increase under oxygen administration calculated on this basis would be 70 per cent for the right hand and 72 per cent for the left hand. The smaller flow for the 7 minutes than for the first 11 minutes of the experiment is probably due to the vaso-motor effect of exposure of the hands for two minutes when they were taken out of the calorimeters by inadvertence, as noted in the protocol. The vaso-constriction in both made the flow more nearly equal in the two hands than at first, and this agrees very well with the practical equality of the percentage increase in the two under the influence of oxygen on the assumption that the oxygen action is a vaso-motor one.

For the 11-minute period when air was breathed after stopping the oxygen the flow fell to 38.29 grammes per minute or 8.80 grammes per 100 cc. per minute for the right hand (with calorimeter temperature 31.32), and to 38.74 grammes per minute or 9.68 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 31.23), almost the same numbers as for the first period of air breathing before oxygen inhalation was begun. It may be pointed out once for all that the continuous small increase in the calorimeter temperature cannot be responsible for the increased flow during the oxygen period since the flow diminishes markedly in spite of a further increase in calorimeter temperature during the subsequent period of air breathing. The room temperature remained constant (at 19.4) throughout the whole experiment so that none of the changes could be affected by this factor.

A week later (experiment of January 27, 1911) the man was again examined in the same way. In the interval he was feeling fairly well and was working at his trade. The similarity in the results, even in the details, is most striking, as is shown in the protocol and the synopsis of the calculated flows which follows it.

S. G. January 27, 1911.

11.41 a.m. Hands put into bath at 29.9°. Room 18.6°. Mouth temperature 37.05. Rectal temperature 37.4. Pulse (sitting) 96.

11.55 Hands put into calorimeters. 3050 cc. water in each.

TIME	A	B	NOTES	TIME	A	B	NOTES
11.54	29.26	29.21		12.23	30.48	30.66	
11.56	29.29	29.26		12.24	30.53	30.70	
12.01	29.44	29.43	Room 19.7	12.25	30.58	30.77	
12.02	29.48	29.47		12.26	30.61	30.80	
12.03	29.52	29.52		12.27	30.68	30.87	
12.04	29.57	29.57		12.28	30.73	30.91	
12.05	29.62	29.61		12.29	30.79	31.00	
12.06	29.66	29.68		12.30	30.86	31.06	
12.07	29.70	29.72		12.31	30.90	31.11	
12.08	29.75	29.80	Room 19.6	12.31-15			Oxygen stopped
12.09	29.80	29.89		12.31-30			Mask taken off
12.10	29.83	29.91		12.32	30.98	31.18	
12.11	29.88	29.99		12.33	31.03	31.22	
12.12	29.91	30.02	Room 19.6	12.34	31.06	31.27	Room 19.9
12.13	29.95	30.07		12.36	31.11	31.30	
12.14	30.00	30.11		12.37	31.14	31.34	
12.15	30.03	30.16		12.38	31.18	31.38	
12.16			Mask put on	12.39	31.22	31.42	
12.16-40			Oxygen started	12.41	31.29	31.50	
12.18	30.16	30.30		12.43	31.34	31.56	
12.19	30.22	30.38		12.45	31.39	31.63	
12.20	30.28	30.43		12.47	31.46	31.68	Hands taken out at 12.47
12.21	30.34	30.52		1.05	31.17		
12.22	30.40	30.60		1.06		31.39	

Volume of right hand 445 cc., of left hand 415 cc.

For 13 minutes during which the subject was breathing air the calculated flow is 31.71 grammes per minute or 7.13 grammes per 100 cc. per minute for the right hand (with mean calorimeter temperature 29.76 and room temperature 19.6), and 37.56 grammes per minute or 9.05 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 29.81). For a

13-minute period during oxygen inhalation the corresponding flows were 44.44 grammes and 9.99 grammes for the right hand (with calorimeter temperature 30.53 and room temperature 19.6) and 48.68 and 11.7 grammes for the left hand (with calorimeter temperature 30.70), an increase of 40 per cent for the right hand and nearly 30 per cent for the left hand. For the subsequent period of air breathing (13 minutes) the flow comes out 31.93 grammes per minute or 7.17 grammes per 100 cc. per minute for the right hand (with mean calorimeter temperature 31.26 and room temperature 19.8) and 33.78 grammes per minute or 8.14 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 31.47).

Analysis of the minute readings in the experiment of January 20, 1911, for the period of air breathing subsequent to oxygen inhalation suggests that the influence of the oxygen continues for a short time after the patient has ceased to breathe it. For the first three minutes of the period the flow comes out 41.95 grammes per minute or 9.64 grammes per 100 cc. per minute for the right hand, and 40.29 grammes per minute or 10.07 grammes per 100 cc. per minute for the left hand. For the last 8 minutes of the period the corresponding numbers are only 36.92 and 8.49 grammes for the right hand, and 38.16 and 9.54 grammes for the left hand. Of course, no great weight could be given to such differences in a single observation especially to those occurring toward the end of a long experiment (see p. 493). But the same thing is indicated in the second experiment of January 13, 1911, in which the oxygen inhalation was stopped a short time before insertion of the hands into the calorimeter, and still more clearly in the experiment of February 3, 1911. Yet the oxygen action is very transient and only outlasts the inhalation by, at most, a few minutes, at least for such periods of inhalation as were employed.

S. G., January 13, 1911. *Experiment 2.* Pulse (sitting) 84. Mouth temperature 36.95.

2.35 p.m. Began to breathe oxygen through a mask.

3.01 Hands put into bath at 30.9°. Room 23.4°.

- 3.08.30 Stopped oxygen and removed mask.
 3.11 Temperature of bath is now 30.7.
 3.12.30 Hands put into calorimeters. 3050 cc. of water in each.

TIME	A	B	TIME	A	B
3.15	30.20	30.11	3.24	30.94	30.83
3.17	30.37	30.30	3.25	31.02	30.90
3.18	30.47	30.40	3.27	31.17	31.03
3.19	30.55	30.48	3.29	31.30	31.16
2.20	30.63	30.56	3.31	31.43	31.29
2.21	30.71	30.62	3.31	Hands taken out of calorimeters	
3.22	30.80	30.70			Room 22.4
3.23	30.88	30.78	3.42	31.29	31.15

Volume of right hand 438 cc., of left hand 408 cc.

For the whole 16-minute period the calculated flow is 56.83 grammes per minute or 12.97 grammes per 100 cc. per minute for the right hand, and 53.42 grammes per minute or 13.09 grammes per 100 cc. per minute for the left hand (with mean calorimeter temperature 30.82 for right, 30.70 for left, and room temperature 23.0). For the first 8 minutes the flow is 59.28 grammes per minute or 13.53 grammes per 100 cc. per minute for the right hand, and 57.32 grammes per minute or 14.05 grammes per 100 cc. per minute for the left hand. For the last 8 minutes the corresponding numbers are 54.38 and 12.41 grammes for the right hand, and 49.61 and 12.13 grammes for the left.

In the forenoon of the same day a measurement (Experiment 1) had been made on this subject while breathing air. He was in the laboratory all day under practically uniform conditions and sitting at rest.

S. G. January 13, 1911. *Experiment 1.* Since the last examination on December 16, 1910, he has been discharged from the hospital and has worked at his trade. He still has some cyanosis. Pulse (sitting) 84. Mouth temperature 36.94. Rectal temperature 37.4.
 11.37 a.m. Hands put into bath at 29.6°. Room 20.0°.
 11.46 Bath is now at 29.7°. Room 21.2°.
 11.48.30 Hands put into calorimeters. 3050 cc. of water in each.

TIME	A	B	TIME	A	B	NOTES
11.46	28.77	28.96	11.59	29.63	29.72	Room 22.2
11.51	28.93	29.10	12.00	29.70	29.81	
11.52	29.03	29.19	12.01	29.78	29.89	
11.53	29.11	29.27	12.02	29.84	29.95	
11.54	29.20	29.36	12.03	29.92	30.02	Room 23.1. Hands out at 12.04
11.55	29.30	29.42	12.04	30.02	30.10	
11.56	29.39	29.50	12.10	29.94	30.01	
11.57	29.47	29.58	12.15	29.87	29.96	
11.58	29.55	29.66				

Volume of right hand 438 cc., of left 408 cc.

For the 13-minute period the flow is 50.52 grammes per minute or 11.53 grammes per 100 cc. per minute for the right hand (with mean calorimeter temperature 29.48 and room temperature 22.3) and 46.94 grammes per minute or 11.50 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 29.60).

While, of course, in general comparison cannot be made for such a purpose as this of observations separated by so long an interval (3 hours), yet the constancy of the external conditions here perhaps permits us to consider the increased flow observed after the inhalation of oxygen in the afternoon (Experiment 2) as corroborating the results of the other experiments.

The experiment of February 3, 1911 also furnishes evidence that the oxygen action persists for a short time after inhalation has been stopped and the subject is again breathing air. It furthermore indicates, as is suggested though less clearly in other experiments, that the maximum effect of the oxygen may require a sensible time (although only a very few minutes) to be reached. In this experiment the subject did not inhale the oxygen through a mask but through a tube held in the mouth. This arrangement was adopted as it was thought it would facilitate the collection of samples of alveolar air for the determination of the carbon dioxide and oxygen tensions, which, as already mentioned, were being simultaneously investigated by Drs. Macleod and Hoover. The carbon dioxide tension in this patient was always

found higher than the maximum given by Haldane for normal persons. It was not obviously diminished by oxygen inhalation.

S. G. February 3, 1911.

10.52 a.m. Hands put into bath at 29.8°. Pulse (sitting) 92. Mouth temperature 37.0. Rectal temperature 37.4.

11.04 Hands put into calorimeters. 3050 cc. of water in each.

TIME	A	B	NOTES	TIME	A	B	NOTES
11.03	29.19	29.19		11.29-15	30.34	30.28	
11.05			Tube put into	11.30	30.39	30.32	
			mouth and	11.31	30.42	30.36	
			he henceforth	11.32	30.47	30.39	Room 18.9
			breathes through	11.33	30.49	30.42	
			tube.	11.34	30.53	30.45	
11.06	29.24	29.22		11.35	30.57	30.50	
11.07	29.29	29.25		11.36	30.61	30.53	
11.08	29.32	29.30	Room 18.25°	11.37	30.64	30.56	
11.10	29.48	29.43		11.38	30.67	30.58	Room 18.9
11.11	29.53	29.51		11.39	30.69	30.61	
11.12	29.60	29.55		11.40	30.71	30.62	Tube removed
11.13	29.67	29.62					from mouth at
11.14	29.71	29.67		11.41	30.73	30.64	11.40
11.15	29.76	29.71	Room 18.3	11.42-15	30.76	30.67	
11.16	29.80	29.72		11.43	30.78	30.68	
11.17	29.81	29.74		11.44	30.78	30.69	
11.18	29.84	29.78	At 11.18 oxygen	11.45	30.79	30.71	Room 19.0
			turned on	11.46	30.80	30.71	
11.19	29.88	29.82		11.47	30.81	30.72	
11.21	29.96	29.90	Room 18.4	11.48	30.83	30.74	
11.22	29.99	29.94		11.49	30.85	30.76	
11.23	30.03	29.98	Room 18.4	11.50	30.87	30.79	
11.24	30.08	30.02		11.51	30.87	30.79	Hands removed
11.25	30.12	30.04					from calorime-
11.26	30.16	30.11					ters at 11.51
11.27	20.22	30.16					
11.28	30.23	30.23	Oxygen turned	12.03	30.69	30.61	Room 18.9
			off at 11.28				

Volume of right hand 440 cc., of left hand 410 cc.

For the first 7 minutes while air is being breathed through the tube the flow is 39.37 grammes per minute or 8.95 grammes per 100 cc. per minute for the right hand (with mean calorimeter

temperature 29.46 and room temperature 18.3), and 36.69 grammes per minute or 8.95 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 29.42). For the last 5 minutes of the air period the flow sinks to 26.02 grammes per minute or 5.91 grammes per 100 cc. per minute for the right hand (with calorimeter temperature 29.76 and room temperature 18.3), and to 24.54 grammes per minute or 5.98 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 29.70). The cause of the diminution was not apparent. It could not have been hypernoea caused by breathing through the tube, although it has been shown that forced breathing diminishes the flow in normal persons.⁴ For no noticeable change occurred in his respiration, and the nose was not clipped. Whatever the cause, the diminution was most likely due to local vaso-constriction. When the oxygen was turned on the flow increased in the first 5 minutes to 29.52 grammes per minute or 6.57 grammes per 100 cc. per minute for the right hand (with calorimeter temperature 29.94 and room temperature 18.4), and to 29.52 grammes per minute or 7.20 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 29.88). In the second 5 minutes of oxygen inhalation the flow still further increased to 36.73 grammes per minute or 8.35 grammes per 100 cc. per minute for the right hand (with calorimeter temperature 30.16 and room temperature 18.4), and to 36.14 grammes per minute or 8.81 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 30.10). The increase, when we compare the second part of the oxygen period with the second part of the air period, is 41 per cent for the right hand and 47 per cent for the left hand. When the oxygen was shut off and air breathing resumed, the flow diminished again, to 33.73 grammes per minute or 7.66 grammes per 100 cc. per minute for the right hand (with calorimeter temperature 30.40 and room temperature 18.9), and to 30.28 grammes per minute or 7.38 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 30.34) for the first 6 minutes. For the second 6 minutes after the resumption of air

⁴ Stewart: *American Journal of Physiology*, 1911, xxviii, p. 190.

breathing the flow sank still further to 27.72 grammes per minute or 6.30 grammes per 100 cc. per minute for the right hand (with calorimeter temperature 30.62 and room temperature 18.9), and to 26.18 grammes per minute or 6.38 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 30.54), flows not materially different from those in the last part of the first air period previous to the inhalation of oxygen.

It is possible that in this experiment the proportion of oxygen in the gaseous mixture inhaled was less than in the experiments with the mask, as the nostrils were not clipped, it being thought that the practice which the patient had had with this method would insure his breathing only through the tube. This, however, was afterwards found not to be the case. Although a large proportion of the gas breathed did pass through the tube some went through the nose. The smaller proportion of oxygen might account for the smaller changes in the flow during the oxygen period. However, these changes are always perfectly distinct and always in the same direction as in the other experiments. It may be due to the same circumstance that the gradual increase of the oxygen action to a maximum could be easily observed in this experiment while it was less clear in the others. It is obvious that if a mixture less rich in oxygen were being inhaled, the change produced by the oxygen on which the augmentation of the peripheral circulation depends would take longer to reach its maximum. The persistence of the oxygen effect for a short time after the oxygen has been stopped is seen in this experiment as in the others. There is, of course, no reason why it should be better seen, since the maximum effect having been once reached whether gradually or quickly, the effect will probably disappear at a rate which is not influenced by the rate at which the maximum was attained.

Ten days after this experiment the patient again presented himself at the dispensary. His cyanosis was more pronounced than at any of the previous examinations, yet his breathing showed no signs of distress. Although the influence of oxygen on the flow was not tested in this experiment, the protocol may be quoted as it brings out the interesting fact that the cyanosis was associated with a greater blood flow (in the hands and without

doubt in the surface generally, whatever may have been the case in the deeper parts) than in any of the other observations, and a flow considerably more copious than in most of the normal persons examined under the same conditions of calorimeter and room temperature. The reflex vaso-constriction produced in one hand by the application of cold water to the other was relatively feeble as compared with the normal, as is generally observed when the vaso-dilatation is already considerable.⁵

S.G. February 13, 1911. He says he is feeling much worse than when last examined, weak and very drowsy. No pain except some headache. He says that about a week ago he slept so long that his family had to awaken him, which was quite unusual. He has been unable to work since. Has some cough. Abundant râles can be heard over both lungs. No dyspnœa. Pulse is more rapid than at previous examination (105 in sitting posture). Face and hands cyanosed. Hands warm. Mouth temperature 37.45. It was only possible to measure the blood flow of one hand as one hand of another patient had to be examined at the same time in the other calorimeter.

2.56 p.m. Put right hand into bath at 29.4°. Room 22.2°.

3.08.45 Put right hand into calorimeter B. 3015 cc. water in calorimeter.

TIME	B		TIME	B	
3.08	29.32	Room 22.0	3.20	30.61	The cold water is now at 14.0
3.10	29.44		3.21	30.67	
3.11	29.56		3.22	30.74	
3.12	29.67		3.23	30.83	
3.14	29.93	At 3.18 immersed left hand in water at 11.2°	3.24	30.93	At 3.23 dried left hand and wrapped it in a towel
3.15	30.09		3.25	31.01	
3.16	30.19		3.26	31.09	At 3.26 put a warm towel round left hand
3.17	30.31		3.27	31.15	
3.18	30.44		3.28	31.23	Took right hand out of B Room 22.4
			3.28-15		
			3.56	30.92	
3.19	30.50		Volume of right hand 440 cc.		

⁵ Stewart: "The effect of reflex vasomotor excitation on the blood flow in the hand," Heart, 1911, loc cit.; Cleveland Medical Journal, loc. cit.

For the 8 minutes preceding the testing of the vasomotor reflex the flow comes out 69.96 grammes per minute or 15.90 grammes per 100 cc. of hand per minute for the right hand (with mean calorimeter temperature 29.94 and room temperature 22.0). For 5 minutes during immersion of the left hand in cold water, the flow in the right was reduced to 51.02 grammes per minute or 11.59 grammes per 100 cc. per minute (with mean calorimeter temperature 30.64 and room temperature 22.2), to increase to 55.34 grammes per minute or 12.58 grammes per 100 cc. per minute (with calorimeter temperature 31.03 and room temperature 22.2) for the 5-minute period after the left hand had been dried and wrapped up.

Before going further it may be well to give in tabular form the flows in S. G. in the different experiments with ordinary air breathing. Naturally the flows are less with the lower room temperatures. Yet on the whole they compare favorably with the flows in M. C., who of all the normal persons examined had habitually the greatest blood flow in the hands.

DATE	MEAN TEMP. OF CALORIMETERS		FLOW PER 100 CC. HAND PER MIN.		ROOM TEMP.
	Right	Left	Right	Left	
16-12-10	30.02		14.66		22.9
13-1-11 a.m.	29.48	29.60	11.53	11.50	23.0
13-1-11 p.m.	30.82	30.70	12.97	13.09	23.0
20-1-11	29.60	29.45	8.51	9.79	19.4
27-1-11	29.76	29.81	7.13	9.05	19.6
3-2-11	29.46	29.42	8.95	8.95	18.3
13-2-11	29.94		15.90		22.0

M. C. Age 23. Normal man

30-11-10	28.00	27.80	10.1	9.4	20.2
22-12-10	29.71	29.46	13.7	12.5	21.1
1-2-11	30.35	30.34	12.66	12.76	22.8
7-2-11	29.19		12.67		19.0
17-3-11	29.94	29.93	11.85	11.29	21.1
18-3-11	29.07	31.44	13.66	13.48	20.5

An essential step in elucidating the mechanism of the oxygen action in this case was to determine whether a similar effect could

be demonstrated in normal persons. Experiments were therefore made on two healthy men, one of whom (M. C.) was known to have a habitually copious blood flow in the hands. The other (E. W.) apparently belongs to a group of healthy persons whose cutaneous flow, at least so far as the hand can be taken as a test, is rather small. It may be said in a word that no increase in the flow was produced in either of these subjects by inhalation of oxygen.

February 6, 1911. M. C., age 23. Laboratory assistant. Height 5 feet, 10 inches. Weight 146 pounds (stripped). Pulse (sitting) 88. Mouth temperature 36.13. Rectal temperature 36.95.

3.20 p.m. Mask put on.

3.32. Hands put into bath at 29.6°. Room 20.7°.

3.42.30 Hands put into calorimeters. 3050 cc. of water in each.

TIME	A	B		TIME	A	B	
3.41	29.03	29.03		4.04	30.08	30.02	
3.44	29.08	29.09	Room 22.3	4.05	30.12	30.05	
3.45	29.13	29.13		4.06	30.18	30.12	
3.46	29.19	29.19		4.07	30.22	30.14	Room 22.3
3.47	29.24	29.21		4.08	30.24	30.16	
3.48	29.29	29.27	Room 22.6	4.09	30.27	30.19	
3.50	29.39	29.37		4.10	30.29	30.22	
3.51	29.48	29.44		4.11	30.30	30.23	At 4-11-20 took off mask
3.52	29.53	29.50		4.12	30.32	30.24	
3.53	29.60	29.58		4.13	30.37	30.28	
3.54	29.65	29.62	Room 22.4	4.14	30.39	30.31	
3-54-30			Attached oxygen tube to mask.	4.15	30.42	30.32	Room 22.3
			Oxygen turned on at 3.55	4.16	30.45	30.34	
				4.17	30.49	30.37	
3.56	29.72	29.67		4.18	30.51	30.40	
3.57	29.77	29.71		4.19	30.53	30.42	
3.58	29.80	29.76		4.20	30.54	30.42	Hands taken out of calorimeters at 4.20
3.59	29.86	29.81	Room 22.3				
4.00	29.90	29.84					
4.02	29.99	29.92		4.33	30.38	30.25	
4.03	30.03	29.97	At 4.03 stopped oxygen. At 4-03-45 disconnected oxygen tube. leaving mask on				
							Volume of right hand 465 cc. Volume of left hand 450 cc.

For the 10-minute period during which he was breathing air through a mask the flow is 37.87 grammes per minute or 8.1 grammes per 100 cc. of hand per minute for the right hand, and 36.10 grammes per minute or 8.0 grammes per 100 cc. per minute for the left hand (with a mean calorimeter temperature of 29.36 for both hands and room temperature 22.5). For the 7 minutes during which oxygen was inhaled through the mask the corresponding numbers are 33.58 grammes and 7.22 grammes for the right hand, and 32.59 grammes and 7.24 grammes for the left hand (with calorimeter temperatures 29.88 and 29.82 respectively and room temperature 22.3). For the first 4 minutes after turning off the oxygen, the subject still breathing through the mask, the flow comes out 36.94 grammes per minute or 8.07 grammes per 100 cc. of hand per minute for the right hand, *i. e.*, exactly the same as in the period before oxygen inhalation, and 33.39 grammes per minute or 7.42 grammes per 100 cc. per minute for the left hand. The flow now began to diminish, as not infrequently occurs toward the end of a long experiment, owing sometimes to venous stasis, in the dependent position of the hand, sometimes to vaso-constriction due to the prolonged immersion. Thus, for the 8 minutes when the subject was breathing air after removal of the mask the flow is only 25.86 grammes per minute or 5.56 grammes per 100 cc. per minute for the right hand (with calorimeter temperature 30.43 and room temperature 22.2), and 22.61 grammes per minute or 5.02 grammes per 100 cc. per minute for the left hand (with calorimeter temperature 30.33). The diminution in the flow was already evident in the last three minutes of the seven-minute period when the subject had ceased inhaling oxygen but was still breathing through the mask. In the observations on E. W. a similar decline in the flow was seen at the end of the experiment when the subject was breathing air without the mask.

February 3, 1911, E. W., Laboratory assistant. Age 30. Height 5 feet, 9½ inches. Weight 185 pounds.

12.10. p.m. Hands put into bath at 30.8°. Pulse 70 (sitting). Mouth temperature 37.2. Rectal temperature 37.6.

12.21.15 Hands put into calorimeters. 3050 cc. of water in each.

TIME	A	B		TIME	A	B	
12.20-30	30.42	30.34		12.40	30.70	30.57	
12.22	30.40	30.31		12.41	30.71	30.59	
12.24	30.42	30.34		12.42	30.72	30.58	
12.25	30.44	30.35		12.43	30.73	30.60	Room 19.2
12.26	30.47	30.37		12.44	30.74	30.59	
12.27	30.48	30.40		12.45	30.76	30.61	
12.28	30.50	30.41		12.46	30.77	30.62	
12.29	30.51	30.42	Room 19.4	12.47	30.78	30.62	
12.30-30			Mask put on	12.48	30.79	30.64	
12.31	30.55	30.44		12.49	30.80	30.64	
12.32	30.57	30.45		12.50	30.80	30.63	
12.33	30.58	30.47		12.51	30.81	30.65	Oxygen stopped and mask re- moved at 12.51
12.34	30.60	30.49	Room 19.4				
12.35	30.62	30.52		12.52	30.82	30.65	
12.36	30.64	30.52		12.53	30.83	30.65	
12.37	30.66	30.53		12.54	30.84	30.66	Room 19.4
12.38	30.68	30.55		12.55	30.845	30.67	
12.39	30.69	30.56	Oxygen begun at 12.39	12.56	30.85	30.68	
				12.57	30.86	30.68	
				12.58	30.86	30.68	
				12.59	30.86	30.67	
				1.00	30.85	30.66	
				1.01	30.83	30.65	Hands taken out of calorimeters at 1.01
				1.13	30.66	30.47	

Volume of right hand 452 cc.

Volume of left hand 445 cc.

The calculated flow for the first period of ordinary breathing (8 minutes) is 16.80 grammes of blood per minute or 3.71 grammes per 100 cc. of hand per minute for the right hand (with a mean calorimeter temperature of 30.46 and room temperature 19.4). For the left hand the corresponding numbers are 15.83 grammes and 3.56 grammes (with calorimeter temperature 30.37). For the next 8 minutes, during which air was breathed through the mask, the flow comes out 18.70 grammes per minute or 4.14 grammes per 100 cc. of hand per minute for the right hand (with calorimeter temperature 30.62 and the same room temperature as before). The corresponding numbers for the left hand are 16.87 grammes and 4.14 grammes (with calorimeter temperature 30.50).

For the 12-minute period of oxygen inhalation the flow, far from being increased as in S. G., is actually diminished. Calculated on the whole 12 minutes, the flow is 14.73 grammes per minute or 3.25 grammes per 100 cc. of hand per minute for the right hand (with calorimeter temperature 30.75 and room temperature 19.2), and 13.42 grammes per minute or 3.02 grammes per 100 cc. of hand per minute for the left hand (with calorimeter temperature 30.61). Analysis of the 12-minute period shows that at no time is any increase in the flow to be observed. Thus, for the first 6 minutes of oxygen inhalation the calculated flow is 15.70 grammes per minute or 3.47 grammes per 100 cc. of hand per minute for the right hand, and 13.88 grammes per minute or 3.12 grammes per 100 cc. per minute for the left hand. For the last 6 minutes of the period the corresponding numbers are 13.76 and 3.04 grammes for the right hand and 12.96 and 2.91 grammes for the left hand. For the first five minutes after removing the mask, the subject breathing air, the flow is 13.61 grammes per minute or 3.01 grammes per 100 cc. per minute for the right hand, and 12.65 grammes per minute or 2.84 grammes per 100 cc. per minute for the left hand, practically the same as during the oxygen inhalation. In the last 5 minutes of the experiment an abrupt decline in the flow occurs, as was seen also in the experiment on M. C. As pointed out in the discussion of that experiment, this decline has no significance for our present question. These 'fag-end' results cannot be properly compared with the preceding part of the experiment.

These results make it clear I think that in the pathological condition the influence of oxygen was not merely an apparent one due to mechanical changes in the respiration associated with the technique of the oxygen inhalation. For the two normal persons inhaled oxygen under the same conditions as the patient. Further, no change in his respiratory movements either in depth or rhythm was noticed during oxygen inhalation. This statement is based both on careful inspection of the chest movements and on respiratory tracings. The total ventilation, estimated by Haldane's body plethysmograph, as determined by Drs. Macleod and Hoover, also remained unaltered. As already men-

tioned, his respiration even when sitting at rest showed group breathing, and this was not affected when he breathed oxygen under the conditions of our experiments.

I do not desire at present to venture on an explanation of the mechanism of the circulatory change produced by oxygen inhalation in this patient, except by offering one or two suggestions. The result of the gas tension estimations, specimens of which are embodied in the following table, afford no obvious clue.

DATE		CO ₂ PERCENTAGE IN MOIST ALVEOLAR AIR			O ₂ PERCENTAGE IN MOIST ALVEOLAR AIR		
		End of Insp.	End of Exp.	Mean	End of Insp.	End of Exp.	Mean
20-1-11 a.m.	Before oxygen.....	6.50	6.69	6.59			
	One min. after stopping oxygen.....	6.60					
20-1-11 p.m.	In body plethysmo- graph.....						
	Before oxygen.....	8.11	8.37	8.24			
	2 min. after stopping O ₂		7.50				
	5 min. after stopping O ₂		7.36				
	15 min. after stopping O ₂		7.56				
27-1-11 a.m.	*Before oxygen.....	7.4			14.7		
	Before oxygen.....	7.9					
	5 min. after stopping O ₂	9.7(?)	8.0		12.0		
17-3-11	Breathing air in ordi- nary way.....	7.87	8.1	7.98			
		8.10	8.2	8.15	12.39	10.19	11.29

*In the afternoon of this day the patient was put into Haldane's body plethysmograph. No change was produced in the pulmonary ventilation during the inhalation of oxygen as shown by the plethysmograph tracings and also by a Wright gas meter (or spirometer) when the patient breathed into valves connected with it. The same result was obtained on the other occasions when this matter was tested. The group type of breathing was well shown by the plethysmograms and was not affected by oxygen.

According to the determinations of Fitzgerald and Haldane⁶ the maximum percentage of CO₂ in alveolar air saturated with watery vapor at 37° C. was 5.86 and the mean 5.16 in the group of men examined. The carbon dioxide tensions of S. G. are therefore invariably above the normal; and in most of the obser-

⁶ Fitzgerald and Haldane: *Journal of Physiology*, xxxii, p. 486, 1905.

vations very decidedly so. In the observations embodied in the table, which are fair specimens of the general results, no diminution of the carbon dioxide pressure was made out in any of the samples collected after oxygen inhalation had been stopped, not even in that collected within one minute in the experiment of 20/1/11 a.m. Nevertheless, as remarked in the discussion of the blood flow measurements, the increase in the flow occasioned by the oxygen persisted for some minutes during the subsequent period of air breathing. In the experiment of the same day in the afternoon the apparent diminution in the CO_2 pressure after oxygen is shown to depend merely upon a temporary increase before the oxygen inhalation is begun by the fact that it remains practically the same 2 minutes, 5 minutes and 15 minutes after the oxygen was stopped. As this was the first time the man had been in the body plethysmograph, it is probable that he was not breathing quite freely at first and therefore gave a higher carbon dioxide pressure than the normal for his condition at that time.

It is not difficult to think of more than one way in which a diminution of an excessive carbon dioxide tension might favor the circulation in the hands. If the increased vaso-constriction in asphyxia be due to stimulation of the vaso-motor center by carbon dioxide, then a reduction of the habitual hypercapnia in this man might very well be associated with an augmentation in the flow, even if, as is shown by the normally copious flow in his hands, his vaso-motor center like his respiratory center was less excitable than normal to carbon dioxide. But the table shows that the carbon dioxide tension was not sensibly diminished by oxygen inhalation. The suggestion, therefore, is that it is not upon a change in the carbon dioxide tension in the blood that the alteration in the circulation depends but rather upon the beneficial effect of the oxygen. This beneficial effect might be *immediate*, upon the metabolism of the vaso-motor center itself or of the heart, or *mediate*, upon the metabolism of the tissues in general, particularly the muscles, by reducing the amount of acid products (lactic acid, for example) formed, or aiding in the combustion of those already present. It would be easy to frame hypotheses to connect both the immediate and the mediate actions of

oxygen inhalation with such changes in the vaso-constrictor center (and also in the vaso-dilator) and with such changes in the force of the heart beat (the rate remained apparently unaltered) as would lead to an increased blood flow in the hands. Possible peripheral effects on the caliber of the small vessels produced by carbon dioxide and other metabolic products⁷ must also not be forgotten. Although the systolic blood pressure in the brachial artery showed no noticeable change (in one experiment it was 130 mm. mercury just before and the same during inhalation of oxygen) the amplitude of the radial pulse was distinctly increased, as shown on the sphygmogram. Before any blood flow measurements had been made, or any opinion formed as to the effect of oxygen inhalation on the flow, a number of men studying the patient's pulse in the ward had called attention to the increased amplitude and diminution of tension in the radial pulse which they detected by palpation, as was mentioned to me by Dr. Hoover.

The result of Hill and Flack⁸ that "inhalation of oxygen allows a man to stand a higher tension of carbon dioxide than is normal before the breaking point," the point at which the breath can no longer be held, "is reached," suggests that the threshold of the vaso-motor (vaso-constrictor) center and of the cardio-inhibitory center may also be raised by oxygen breathing. They believe their experiments show "the remarkable fact that a high oxygen tension moderates the effect of both an abnormally low and high tension of carbon dioxide." The lowest tension of carbon dioxide observed by them with forced breathing of oxygen was 1.47 per cent and the highest (during exertion with the breath held) 11.18 per cent before the breaking point was reached. Their results on the influence of oxygen in combating the unpleasant effects associated with forced breathing of air are also suggestive, although there was no forced breathing in our observations on S. G. For they show that certain symptoms, produced by the washing out of carbon dioxide in forced breathing of air

⁷ Bayliss, loc. cit.

⁸ Loc. cit.

and clearly connected with the diminished blood flow which I demonstrated in two healthy men during forced air breathing,⁹ are relieved by oxygen. If these symptoms—numbness and the sensation of pins and needles in the limbs, acceleration and enfeeblement of the pulse and fall of systolic blood pressure during inspiration, a feeling of constriction in the head or neck and a partly dazed mental condition—are associated with diminution in the blood flow and are relieved by oxygen, or do not appear with forced breathing of oxygen, which also causes washing out of carbon dioxide, then it is demonstrated that one condition which produces such enfeeblement of the circulation as can be detected in the hands by our method is relieved by oxygen. In other words, in this condition oxygen must cause an increased circulation. If it does so in one abnormal condition, it may do so in another, viz: in the condition seen in our cyanotic patient, and probably in the same or in a similar way. When oxygen is administered as a therapeutic agent in other conditions associated with cyanosis, as in pneumonia, we can hardly be far wrong in assuming that a part of the beneficial action, not excluding the subjective feeling of relief, is due to a diminution of the peripheral vascular resistance. It is stating the same thing in a different way to say that an element in air hunger may be an increase in this resistance conditioned by an under-oxygenation of the blood, which throws an extra strain upon the heart and conspires with the diminished oxygen tension in the blood to heighten the asphyxia of the tissues, including the heart itself, by cutting down their supply even of such poorly oxygenated blood as is available. The beneficial effects on the circulation of so-called oxygen baths, in which nascent oxygen is liberated by the action of catalysing substances, is certainly in part due to inhalation of the oxygen, rising from the water, as Tornai holds, who saw dyspnoea and cyanosis quickly vanish under the influence of the oxygen bath. But there is no reason to question the possibility of Winternitz's idea¹⁰ that the effect may also be due in part to a stimu-

⁹ American Journal of Physiology, loc. cit.

¹⁰ *Blaetter für Klin. Hydrotherapie*, 1907, No. 1.

lating action on the cutaneous nerves and a clonic rhythmical contraction of the cutaneous musculature, probably produced mechanically by the innumerable small oxygen bubbles. According to him and to Schnuetgen¹¹ a beneficial effect is thus exerted on the cutaneous circulation while at the same time there is dilatation of the muscular vessels. Frankl¹² found the bath useful in allaying the vasomotor disturbances which occur in women at the menopause and in reducing abnormally high arterial blood pressures.

SUMMARY

1. In a man with pulmonary emphysema, chronic bronchitis, and recurring cyanosis not associated with dyspnoea, the blood flow in the hands was increased by oxygen inhalation by an amount varying from 30 per cent to 70 per cent of the combined flow in the two hands in experiments made on different days during a period of two months.

2. In general, a greater percentage increase under oxygen inhalation was obtained when the initial flow was relatively small than when it was large. In this patient the normal flow (without oxygen inhalation) was habitually large, quite as large as in any of the normal cases studied and larger than the average of the normal cases.

3. The action of oxygen on the blood flow was not associated with any sensible change in the respiratory movements.

4. The action of oxygen on the blood flow was not associated with any certain change in the alveolar carbon dioxide tension.

5. It was therefore conditioned in some way by the increased alveolar oxygen tension. Possible ways in which this could affect the flow are discussed.

6. Oxygen inhalation produced no sensible change in the rate of the blood flow in the hands of two normal men.

¹¹ Therapie d. Gegenwart, April 1907.

¹² Zeitschrift f. Physik. Therapie, August 1908.

FURTHER DATA RELATING TO THE USE OF CERTAIN ANTIMONIAL COMPOUNDS IN THE TREATMENT OF EXPERIMENTAL TRYPANOSOMIASIS

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In a recent publication¹ the results obtained by the authors up till July 1, 1910, in the treatment of experimental trypanosomiasis by means of sodium antimony thioglycollate and the triamide of antimony thioglycollic acid were given. A final report of the subsequent history of treated animals which were living at the time of that writing (July 1, 1910,) is here presented.

Of 158 infected rats which were subjected to treatment, 55 were living on July 1, 1910. These were left with the laboratory attendant during the summer months, 15 being still alive on October 1, 1910. Although the blood of many of these rats dying during the summer was examined just prior to death, in no instance were trypanosomes found, nor have they been found at any time in the blood of any of these animals which have been under personal observation since October 1.

In the Proceedings of the American Society of Pharmacology and Experimental Therapeutics appears a brief report² of the condition of these animals up to December 31, 1910. At that time 9 were still living, the subsequent data relating to these animals is presented in Table I. The record of another animal living at that time but not recorded in the last report is also included.

¹ This Journal, vol. ii, p. 101. 1910.

² This Journal, vol. ii, p. 396.

TABLE I

DISEASE	DATE OF INFECTION	HOURS ELAPSING BEFORE INSTITUTE TREATMENT	NUMBER OF INFECTIONS	DOSE GIVEN	DRUG USED	CONDITION	LIVED AFTER INFECTION	LIVED AFTER LAST TREATMENT
				<i>mgs.</i>			<i>days</i>	<i>days</i>
Surra of Mauritius	3-28-10	48	12	4	Sod. ant. thio-glycollate	Alive	413	340
Surra of India	5- 1-10	72	3	6	Triamide of ant. thioglycoll. acid	Dead	296	232
Surra of Mauritius	5-31-10	96	3	5	Sod. ant. thio-glycollate	Lost	Final result not known	
Dourine.....	3- 4-10	96	12	5	Triamide.	Dead	413	319
Surra of India	2- 9-10	48	14	3	Sod. ant. thio-glycollate.	Dead	325	217
Nagana.....	2-19-10	120	18	5	Triamide	Dead	323	215
Nagana.....	1- 4-10	24	4	1	Sod. ant. thio-glycollate	Dead	423	418
Nagana.....	12-21-09	48	3	2	Sod. ant. thio-glycollate	Dead	496	476
Nagana.....	1- 4-09	24	1	3	Sod. ant. thio-glycollate	Dead	443	442
Surra of Mauritius	4-24-11	96	2	3.5	Sod. ant. thio-glycollate	Dead	363	353

Of the total number treated

37 lived more than 100 days after infection.
 21 lived more than 150 days after infection.
 12 lived more than 200 days after infection.
 10 lived more than 250 days after infection.
 7 lived more than 300 days after infection.
 5 lived more than 350 days after infection.
 4 lived more than 400 days after infection.

Only one is still living at present, 413 days since infection and 340 days since the last treatment.

Nine rats lived more than 200 days, 6 more than 300 days, and 3 more than 400 days after the cessation of treatment.

Of seven infected rabbits under treatment only one is still living, 421 days having elapsed since its infection and a year since the last treatment. Treatment was started on the fourteenth day after infection. Of the other rabbits treated one lived 203 and another 236 days.

A bitch infected with nagana, November 19, 1909, was treated with an arsenic preparation, dimethyl-amido arsen oxide, two weeks later at which time its blood contained numerous trypanosomes and the animal was blind as a result of a keratitis, set up by the disease. The trypanosomes disappeared from the blood and the keratitis quickly cleared up. She has remained in perfectly normal condition, repeated inoculations of her blood into rats failing to produce the disease. One month ago she littered and has been in absolutely normal condition since. On May 15, 1911, she was *exceedingly active, fat and high spirited*. On May 16 she was found dead in her cage, the autopsy revealing nothing but marked congestion of the lungs. The spleen was small, hard and anaemic, which is in striking contrast to the enlarged spleen of trypanosomiasis. No trypanosomes could be found in her blood, an immediate examination being made, and blood inoculated into rats failed to produce the disease. Certainly this dog was permanently cured of its nagana infection and the sudden death was due to some unknown cause.

For a further opportunity of investigating the therapeutic value of other antimonial compounds in nagana we are indebted to Prof. J. Bishop Tingle of McMaster University, Toronto. In the following experiments an antimony compound of succinic acid kindly furnished by him was used, in the preparation of which succinic acid, KOH and antimony oxide were combined in the following molecular ratio 1:1:0.5.

This salt is readily soluble in water or physiological saline yielding a clear colorless solution which is neutral in reaction. The solutions first used contained 5 mg. to the cubic centimeter. These were found to be markedly irritant locally when injected subcutaneously. Thereafter solutions containing 2.5 mg. to the cubic centimeter were employed. These were also markedly irritating, sloughing occurring at the point of injection in numer-

ous instances. In this respect this antimony preparation is decidedly inferior to the thioglycollic acid compounds used in our previous work, which can be safely administered in solutions containing sodium antimony thioglycollate 5–10 mg. to the cubic centimeter, and the triamide of antimony thioglycollic acid 10–15 mg. to the cubic centimeter without any evidence whatever of local irritation.

From the standpoint of toxicity it is also much inferior. Whereas 5 to 6 mgs. of sodium antimony thioglycollate pro 100 gm. weight and 10 mgs. of the triamide pro 100 gm. weight are readily tolerated by rats, 3 mg. pro 100 gm. weight is the outside limit of toleration for the antimony salt of succinic acid. When repeated doses are administered it is safer to give only 2 mg. pro 100 gm. weight.

This new antimony salt however is just as efficient in causing a temporary disappearance of trypanosomes at least as are the other compounds, the time occupied in accomplishing this being the same, one and one-half to two hours in either instance.

Thirty-one rats were infected with *T. brucei*³ on March 15, 1911. Thirty hours after infection occasional trypanosomes being found in the blood, ten of these received treatment subcutaneously with the antimony succinate preparation, 3 mg. pro 100 gm. weight. Five of these showed a relapse by March 23 and all by March 28. The antimony compound in the same dosage was repeated, as the relapses occurred, three dying in the meantime. The treatment was repeated on March 31, the trypanosomes again disappearing although only 2 mg. pro 100 gm. weight was employed. Relapse occurred in each instance or death from toxicity, the last rat dying on April 6.

On March 17, 1911, 50 hours after infection, 12 rats were subjected to the same treatment, 3 mg. to 100 gm. weight, this being repeated on the 20th and the 22d. Four died without relapse before March 26, 1911, toxicity in all probability being responsible for deaths. On the 29th, two relapses having occurred, treatment was repeated, 2 mg. pro 100 gm. weight being employed

³ We acknowledge with pleasure our indebtedness to Dr. Novy of Michigan University, and to Dr. Terry of the Rockefeller Institute for Medical Research for the strains of nagana used in this investigation.

instead of 3 mg. Five of these animals died during the following week. Of the remaining three, two suffered from relapses, one dying on April 16 and the other on April 29. One is yet alive two months after the date of infection, no trypanosomes being demonstrable.

Of six rats treated on March 18, 1911, 75 hours after infection, at a time when the blood was swarming with trypanosomes, one died within 12 hours of the injection, 3 mg. to 100 gm. weight. All of these animals were terribly depressed by the drug, lying on their sides and panting very forcibly, these symptoms persisting for about 1 hour. The same dose was repeated on March 22, no relapses occurring in the meantime. One relapse being encountered on March 27, the rats were again treated (2 mg. to 100 gm.) on that date and again two days later. On April 4, 1911, three relapses were found, the animals receiving another treatment. Relapses quickly followed, the last rat dying on April 15, 1911.

An attempt was made to ascertain if moderate doses repeated at frequent intervals for a period of ten days or two weeks, would prove more efficient. Three rats of this same series, infected on March 15, were given 2 mg. pro 100 gm. of the antimony compound on the 18th, 19th, 23d, 27th and 29th of March. The first relapse appeared on April 4, the treatment being then repeated. Two animals showed a relapse, one on the 10th and the other on the 12th, the latter dying on the 15th.

It therefore is apparent that although this antimony salt of succinic acid is capable of causing the disappearance of trypanosomes, yet in the vast majority of cases its action is of very short duration. It is irritant locally, more toxic than the sodium antimony thioglycollate or the triamide of antimony thioglycollic acid and much less efficient in the treatment of experimental trypanosomiasis.

The excellent results which have been obtained in the treatment of experimental trypanosomiasis by these antimony compounds of thioglycollic acid, their comparative low toxicity and the fact that they are non-irritant locally in comparatively concentrated solution should obtain for them a trial in the treatment of sleeping sickness in the infected districts.

THE LIVER IN ITS RELATION TO ANAPHYLACTIC SHOCK

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In a recent publication, Manwaring¹ reported some interesting experiments on the mechanism of anaphylactic shock in dogs. He demonstrated the fact that in the absence of some of the abdominal organs, especially the liver and the intestine, a shock cannot be produced. Manwaring does not commit himself to a definite explanation of this observation, although he is inclined to believe that the liver, after the first injection of the antigen, contains a specific antibody which, to a smaller extent, is also fixed in other tissues. The anaphylactic reaction possibly consists in a protein digestion. The resulting toxin (anaphylatoxin) possesses the characteristic vasodilating properties, which cause finally the symptoms of anaphylactic shock in the dog.

On account of the prime importance of this discovery it seemed to us well worth while to repeat the experiments quoted with a somewhat improved technic.

The most striking symptom of anaphylactic shock in dogs consists in an abrupt fall of blood pressure, which is due to a peripheral action of the toxin on the blood vessels, especially those of the splanchnic area.² In our work we have considered this symptom a criterion of the existence of anaphylactic shock. Special

¹ Bull., Johns Hopkins Hosp. 1910, xxi, 275. Zeitschr. f. Immunitätsforsch., 1910, viii, 1.

² Biedl and Kraus, Wien. klin. Woch., 1909, xxii, 363. Pearce and Eisenbrey. Jour. Infect. Dis., 1910, vii, 565.

attention was paid also to the time of blood coagulation and to any obvious respiratory changes.

The difficulty of excluding the liver from the general circulation was effected by Manwaring by introducing into the portal vein a cannula which was connected with a second cannula introduced into the external jugular vein, thus allowing the portal blood to flow directly to the heart; hirudin had been previously injected to prevent coagulation of the blood. As stated by Manwaring, hirudin is highly toxic to dogs and it seemed to us necessary to eliminate this poison in our work. By making an Eck fistula,³ combined with ligation of the portal vein near the hilus of the liver and by temporarily clamping the hepatic artery, it was made possible to test the animals under practically normal conditions. Some of our dogs were sensitized before, some after the operation for the Eck fistula. The results of these experiments are illustrated by the following protocols:

No. 4410. December 2, 1910. Bull dog. Weight 10 kilo

Ether anaesthesia. Eck fistula cut. Ligation of portal vein near the hilus of the liver. Prompt recovery. Beef and bone diet.

December 6. Turned out into yard.

December 22. Weight 9.2 kilo. Wound healed. Animal in good condition.

Subcutaneous injection of 20 cc. normal horse serum.

January 23, 1911. Weight 11 kilo. had been starved for 12 hours.

3-30 p.m. Light ether anaesthesia. Incision along linea alba. Branch of femoral artery connected with mercury manometer and kymograph. Femoral vein used for injection of horse serum. Hepatic artery clamped by means of bull-dog clamp.

Effect of injection of horse serum on blood pressure:

Hepatic artery clamped: 160 mm.

Hepatic artery released: 90 mm.

Ether removed and wound closed at 4.15 p.m.

5-15 p.m. Dog conscious, but unable to walk.

January 24. Found dead.

³ Bernheim, Homans and Voegtlin, *The Jour. of Phar. and Exp. Therapeutics*, Vol. 1, No. 5, 1910.

Autopsy: considerable hemorrhage in peritoneal cavity. Blood dark color, hemolized to some extent, does not clot. Eck fistula 3 mm. in length. No collateral circulation of liver.

Cause of death: hemorrhage, due to loss of blood from separated adhesions.

No. 3610. Large male dog. Weight 13.7 kilo.

November 12, 1910. Had been starved for 24 hours.

Ether anaesthesia. Eck fistula made. Portal vein ligated near hilus of liver.

December 22. Dog in excellent condition. Weight 14.2 kilo. Subcutaneous injection of 30 cc. of normal horse serum.

January 26, 1911. Weight 14.7 kilo. Tested for anaphylactic reaction. Light ether anaesthesia. Hepatic artery clamped. Branch of femoral artery connected with kymograph. Horse serum injected into femoral vein (20 cc.).

Hepatic artery clamped: Blood pressure 120 mm.

Hepatic artery released: Blood pressure 86 mm.

Dog recovers from anaphylactic shock very slowly. Is all right following day and is still living at present time (May 28th).

No. 3410. Black female dog. Weight 11 kilo.

November 5, 1910. Subcutaneous injection of 25 cc. normal horse serum.

November 6. Ether anaesthesia. Eck fistula made. Recovers soon after operation. Fed on beef, bread and bones.

November 9. Turned out into yard.

December 17. Animal in excellent condition. Had been starved for 18 hours. Weight 11.2 kilo. Light ether anaesthesia. Hepatic artery dissected out and clamped by means of bull-dog clamp. Branch of femoral artery connected with kymograph. Femoral vein used for injection of horse serum (10 cc.).

Hepatic artery clamped: Blood pressure 170 mm.

Hepatic artery released: Blood pressure 90 mm.

Dog dies after ten minutes with symptoms of asphyxia and great fall of blood pressure.

Autopsy: Liver dark red, congested. Blood clots very slowly and forms only small coagulum. Lungs collapsed.

These experiments conclusively demonstrate the fact that the liver is essential for the development of anaphylactic shock. We have never observed the slightest fall in blood pressure at the time of the second injection of the horse serum when the liver was excluded from the general circulation. We, therefore, cannot confirm the statement of Manwaring, that an atypical shock may develop independently of the liver. Whether this difference is due to the fact that Manwaring used hirudin in his experiments we are not able to state, as the number of our successful experiments (six) is too small for such an assertion. It may be interesting to note that in three of the dogs which were sensitized after the operation for the Eck fistula, no shock developed at the time of the second injection of the horse serum. One might think that in these cases the anti-anaphylactic mechanism described by several investigators had rendered the horse serum phylactically inactive before it had a chance to reach the liver and give rise to an antibody. This consideration is, as may be strongly emphasized, of a hypothetical nature. Still it may have some value as a working hypothesis in devising further experiments.

The question now arises, What is the mechanism by means of which the liver develops the anaphylactic shock? One might think of two possibilities. First, the vasodilating substance is produced outside of the liver and acts secondarily on the liver capillaries, causing a dilation in this area which is sufficient to account for the tremendous fall in arterial pressure. This explanation is not very plausible for the following reason: In sensitized dogs in which the liver is excluded from the general circulation, no shock develops after the second injection of the antigen if a certain period (5 to 10 minutes) has elapsed between the time of injection and the releasing of the liver ligatures. Had the anaphylactic toxin been produced outside of the liver (in any considerable amount) it certainly should act after it gets access to the liver capillaries, unless it is inactivated in some way or another. One might also expect from the work of Schultz,⁴ that some symptoms (peristalsis, defecation, urination) would arise, as this investiga-

⁴ This Journal, vol. I, p. 549, 1909-10, and vol. II, p. 221, 1910.

tor had shown that isolated smooth muscles of sensitized animals (guinea pigs) gives a contraction on being brought into contact with the antigen used for the sensitization. As a matter of fact, none of those symptoms can be observed. It is, therefore, very doubtful that the liver plays only a secondary rôle in anaphylactic shock in the sense that the anaphylatoxin is produced mainly in the circulating blood and fixed tissues (other than the liver) and that it acts, after its production in specifically dilating the liver capillaries.

In this investigation we have tested another possibility. Manwaring mentioned among other explanations, that anaphylactic shock might be due to a liberation of some substances normally present in the cells of the liver and intestine, or appearing in these tissues only after the sensitization with protein.

The liver of dogs, killed under light ether anaesthesia by bleeding from the carotid artery, was freed from all traces of blood by perfusion from the portal vein with a 0.8 per cent NaCl solution, kept at a temperature of 37° C. Saline extracts (5 cc.) of such organs injected into normal dogs caused a temporary, but considerable, fall of blood pressure (60 mm.). There seemed to be no qualitative difference in the action of liver extracts of normal and sensitized animals.

In some of the animals which were injected without the use of an anaesthetic, defecation and urination followed the injection of the extract, besides the appearance of muscular weakness and nervous depression, symptoms found in anaphylactic shock.

The activity of the liver extract is lost by boiling. The filtrate from the coagulum is lacking in all the characteristic properties.

From this we may conclude that in all probability the active substance is either of protein or enzymatic nature. Further experimental work along these lines, which is in progress, is necessary to decide whether or not anaphylactic shock really depends on the liberation of a definite substance by the liver.



A STUDY OF THE ANTISEPTIC AND THE PHARMACOLOGIC PROPERTIES OF META-CRESOL ACETATE

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I. INTRODUCTION

Phenol and the cresols have been used as antiseptics for many years. They are not suitable intestinal antiseptics, however, because, being quite soluble, they are readily absorbed from the stomach and, in any but very dilute solutions, are extremely corrosive. Their external use, also, is dangerous. Absorption of phenol or a cresol from a large area is apt to result in severe intoxication and the application of aqueous solutions in dressings, etc., to the extremities is frequently followed by gangrene. Consequently, these substances are used externally to a much smaller extent than was formerly the case and are employed internally chiefly in the form of compounds such as salol. Such compounds are but sparingly soluble in water and are not materially decomposed in the stomach. They are gradually broken down in the intestine, where they yield the active constituents. These are produced in such low concentrations as to be almost non-irritant.

Of the three isomeric cresols and phenol, meta-cresol is the least toxic. This has been reported by a number of observers. Thus, Tollens¹ gives the data summarized on the next page.

¹Tollens: Arch. f. Exp. Path. and Pharm., 1905, lii, p. 220.

	LETHAL DOSE PER GRAM		LETHAL DOSE PER KILO
	Frog	Mouse	Cat
	<i>mg.</i>	<i>mg.</i>	<i>gm.</i>
Phenol.....	0.10	0.35	0.09
Para-cresol.....	0.15	0.15	0.08
Ortho-cresol.....	0.20	0.35	0.09
Meta-cresol.....	0.25	0.45	0.12

Meili² reported that meta-cresol was less toxic to rabbits than either phenol, ortho-cresol or para-cresol. Binet,³ who used rats and guinea pigs, found that meta-cresol is less toxic than either of its isomers.

Although less toxic to animals, meta-cresol has a greater bactericidal action than either of its isomers or phenol. This has been established by the researches of many investigators, among whom may be mentioned Hammer,⁴ Seybold⁵ and Hammerl.⁶

The great toxicity and corrosive action of the phenols seem to be influenced by the hydroxyl group attached to the benzol ring. If a phenol is modified by the introduction of a radical which eliminates the hydroxyl group, the toxicity is very much diminished. It appears, therefore, that if meta-cresol could be modified in a manner that would diminish its toxic and corrosive properties without interfering with its germicidal activity, the product might be of considerable therapeutic value.

Several years ago, Dr. Nathan Sulzberger, of this city, began an effort to produce, from meta-cresol, a compound that would possess the strong germicidal and analgesic action of the phenols, but which would lack their irritating and escharotic properties. Meta-cresol acetate was the outcome of that investigation. Dr. Sulzberger submitted the results of his work to Dr. Gies, with whose advice I have performed the experiments described in this paper.

²Meili: Vergleichende Bestimmung der Giftigkeit der drei isomeren Kresole und des Phenols. Berne, 1891.

³Binet: Rev. med. de la Suisse romande, 1895, p. 561.

⁴Hammer: Arch. f. Hyg., 1891, xii, p. 359.

⁵Seybold: Z. f. Hyg., 1898, xxix, p. 377.

⁶Hammerl: Hyg. Rundschau, 1899, ix, p. 1017.

II. EXPERIMENTAL

General Physical and Chemical Properties of Meta-Cresol Acetate

Physical properties. Meta-cresol acetate is a colorless, oily liquid, which boils at 212° (uncorrected) and possesses an agreeable odor. Its specific gravity at 26° (H_2O at $4^{\circ} = 1$) is 1.048. It is insoluble in water and glycerol, but is freely miscible with common organic solvents, such as alcohol, ether, chloroform, petroleum ether and benzol.

Chemical stability. Meta-cresol acetate is quite stable. A sample that had been about the laboratory for over five months had the same boiling point as a fresh preparation. It gives only those tests for meta-cresol in which strong hydrolyzing reagents are used.

Resistance to hydrolysis. In order to estimate the rapidity with which meta-cresol acetate may be hydrolyzed under various conditions, 10 cc. were added to each of three bottles containing 500 cc. of 0.5 per cent hydrochloric acid solution, 1 per cent sodium carbonate solution and distilled water, respectively, all at 40°C . Ten cubic centimeter samples of the acid and alkaline solutions were removed immediately after the addition of the meta-cresol acetate and at intervals thereafter. Each was titrated with $\frac{N}{5}$ ammonium hydroxid or sulfuric acid solution, with Congo red as the indicator. The distilled water was tested for acidity and with ferric chlorid solution for free cresol. The ferric chlorid reaction was negative throughout, probably because the meta-cresol formed was kept in solution in the excess of the acetate. The figures obtained in these experiments are given in Table I.

In the acid and alkaline solutions, meta-cresol acetate was gradually decomposed. Hydrolysis by distilled water proceeded very slowly. Apparently, then, meta-cresol acetate would be decomposed very slowly under physiological conditions.

Action on protein. When thoroughly shaken with a fairly concentrated solution of egg-white, meta-cresol acetate coagulated some of the protein. Under these conditions the droplets

TABLE I

Hydrolysis of meta-cresol acetate in 0.5 per cent HCl solution, 1 per cent Na₂CO₃ solution and distilled water
Cubic centimeters of $\frac{N}{5}$ acid or alkalin solution required to neutralize 10 cc. of the liquid

	0.5 PER CENT HCl	1 PER CENT Na ₂ CO ₃	H ₂ O (ACIDITY)
<i>hours</i>			
0 (Control)	7.1	11.0	none
1	7.1	10.6	none
2	7.4	10.4	none
3	7.3	10.2	none
5	7.3	10.1	none
8	7.5	9.8	none
24	7.7	9.6	none
31	8.05	9.6	none
51	8.3	9.3	none
75	8.6	9.0	none
120	8.8	9.2	none
<i>Days</i>			
6	9.0	9.2	none
8	9.4	8.8	none
10	9.6	8.7	none
14	12.8	8.5	none
17	13.1*	8.3	none
22		8.3	slight
30			0.3
35			0.3
193			0.8
232			0.8
254			1.8

*Completely dissolved.

of meta-cresol acetate were coated by coagulated protein, which prevented their coalescence. The greater part of the protein, however, was not coagulated even after contact with the acetate, with frequent shaking, for four days.

Antiseptic Action

Meta-cresol acetate acts as a powerful antiseptic. In one experiment 0.02 cc. in 100 cc. of peptone broth kept at 38° prevented putrefaction for over seventy-two hours. In two flasks,

each containing 100 cc. of such broth, to which had been added, respectively, 0.04 and 0.08 cc. of meta-cresol acetate, there was no appreciable bacterial growth after two months in the incubator. In another series of experiments 0.06 cc. added to 100 cc. of peptone broth checked the multiplication of bacteria to such a degree that prior to the fifth day of incubation, there was no evidence of film formation. Under these conditions 0.08 cc. prevented putrefaction indefinitely.

Comparative tests were made with *meta-cresol*. It was found that 0.07 cc. in 100 cc. of peptone broth checked putrefaction for five days and 0.1 cc. prevented it indefinitely. In this respect, therefore, meta-cresol was found to be as active as the free cresol.

The broth that had been preserved with meta-cresol acetate did not give a test for cresol with ferric chloride. Apparently, then, the antiseptic properties of the acetate were not due to its decomposition into acetic acid and meta-cresol.

Urine to which a little meta-cresol acetate had been added did not decompose. The acetate, however, was slowly hydrolyzed, the acidity of the urine increasing slightly from day to day.

Effects on Animals

Experiments on frogs. Subcutaneous injection. Meta-cresol acetate being insoluble in water and separating from it very readily, it was impossible to use an aqueous or saline emulsion in this work. In the experiments upon frogs a mixture of glycerol and water having the same density as meta-cresol acetate was used. Such a mixture contains about 20 per cent of glycerol and, when shaken vigorously with meta-cresol acetate, forms a fairly stable emulsion. A control injection of the maximum volume of the water-glycerol solution which was used as a carrier in these experiments was without effect. The injected emulsion contained 2.01 grams of meta-cresol acetate in 100 cc. Since the lethal dose of meta-cresol acetate was found to be considerably greater than that calculated from the figures given by Tollens (p. 2), 1.635 gram of meta-cresol was made up to 100 cc. with 20 per cent glycerol solution and portions of this liquid were

injected into a number of frogs. When injected into a dorsal lymph sac both meta-cresol acetate and meta-cresol, in such media, produced the usual symptoms of phenol poisoning. Over-excitability and convulsions were more marked in the frogs that were given the acetate, and paralysis was the chief symptom in those that received the free cresol. The essential data are summarized in Table II.

TABLE II
*Comparative toxicity of meta-cresol and its acetate introduced subcutaneously.
Experiments on frogs*

	META-CRESOL ACETATE		META-CRESOL
	Amount Administered per Gram	Meta-cresol Equivalent per Gram	Amount Administered per Gram
	mg.	mg.	mg.
Maximum dose not followed by symptoms...	0.207	0.149	0.147
Minimum dose followed by symptoms.....	0.258	0.186	0.199
Maximum dose followed by symptoms but not terminating in death..	0.614	0.442	0.486
Lethal dose.....	0.469*	0.338	0.294*
Lethal dose.....	0.588†	0.423	0.287‡
Lethal dose.....	0.714§	0.514	0.419¶
Lethal dose.....	0.637°	0.458	0.427°

*Death occurred between nine and twenty-four hours after injection.

†Death in two hours.

‡Death in eight hours.

§Death in one and one-half hours.

¶Death in four hours.

°Death within one hour.

Considerable variation in the resistance of frogs to the influence of these substances was observed but the results show that the poisonous action of meta-cresol acetate on frogs is approximately equal to that of its meta-cresol equivalent.

Experiments on dogs. Administration per os. A number of dogs received meta-cresol acetate and also meta-cresol by mouth. Each dose was given in gelatin capsules concealed in balls of hashed meat. The animals were found to vary greatly in their resistance as is shown by the data summarized in Table III.

TABLE III

Comparative toxicity of meta-cresol and its acetate given by mouth. Experiments on dogs

	META-CRESOL ACETATE		META-CRESOL
	Amount Administered per Kg.	Meta-cresol Equivalent per Kg.	Amount Administered per Kg.
	gm.	gm.	gm.
Maximum dose not followed by twitching of muscles.....	0.449	0.323	0.377
Minimum dose followed by twitching of muscles.....	0.442	0.318	0.377
Maximum dose followed by twitching of muscles but not resulting in death.....	0.733	0.528	0.810
Minimum lethal dose....	0.836*	0.602	0.794†
Lethal dose.....	1.096‡	0.800	0.907§
Lethal dose.....	1.126¶	0.810	
Lethal dose.....	0.998¶	0.718	

*Death in forty-eight hours.

†Death in two hours.

‡Death in twenty-six hours.

§Death in six hours.

¶Death between nine and twenty-four hours.

From the figures in Table III it seems that meta-cresol acetate is somewhat more toxic than its chemical equivalent of meta-cresol. At the same time it should be noted that some of the results do not warrant this conclusion. Probably the number of dogs used (eight for the acetate and seven for meta-cresol) was not sufficient to rule out the element of uncertainty.

The symptoms usually observed after administration of meta-cresol acetate were drowsiness, followed in about one hour, if the dose was sufficiently large, by twitching of the muscles and by convulsions. When a non-lethal dose was administered, these effects disappeared in a few hours and recovery was rapid. Otherwise the animal remained in a rather apathetic state until death ensued. At autopsy the chief findings were several small fresh

ulcers in the small intestine, the mucosa of which was usually congested; and possibly somewhat more marked congestion and ulceration of the mucosa of the large intestine.

The dog that died forty-eight hours after the administration of meta-cresol acetate presented a hemorrhagic area, about two inches in diameter, on the outside of the fundic portion of the stomach. The liver resembled the so-called "nutmeg liver;" kidneys and spleen showed a passive congestion.

The dog that received 1.096 gram of meta-cresol acetate per kilo, was found dead the following morning. A number of small subcutaneous and mesenteric hemorrhagic areas were discerned. The liver and kidneys were dark brown and the gross markings were unusually distinct.

It was rather surprising to find that in no case was the gastric mucosa congested or affected in any perceptible manner.

Two dogs were made the subjects of continued dosage per os. One, weighing 14 kilos, received 15.2 grams of meta-cresol acetate in three days, none for five days, and then a total of 44 grams during the next ten days. In the first period the doses were increased from day to day. In the second period, which was ten days in length, the meta-cresol acetate was given daily except on the fourth, fifth and ninth days. No ill effect was noted other than a disposition to vomit any considerable volume of food given less than seven or eight hours after administration of the meta-cresol acetate.

Another dog, weighing 4.2 kilos received 2.561 grams of meta-cresol acetate in five days and 8.181 grams in the next seven days. In each period the daily doses were almost uniform. The day after the acetate was last given the dog was killed with chloroform. There were five small, old, healed ulcers such as are frequently seen in dogs, in the small intestine. Otherwise nothing abnormal was found.

Intravenous injection. Under cocaine anesthesia the right femoral vein of a fox terrier weighing 7 kilos was exposed and connected by means of a cannula and rubber tube, to a burette containing physiological salt solution. At intervals por-

tions of a solution of meta-cresol acetate in olive oil⁷ (26.2 grams of meta-cresol acetate per 50 cc. of solution), were injected into the rubber tube near the cannula and then washed into the circulation with 5 or 10 cc. of the saline solution. Injection of 0.5 cc. of the oily liquid caused the heart to beat more rapidly almost immediately, the pulsations increasing to over 150 per minute and decreasing slowly to 130. Three minutes later 0.5 cc. more were injected. This was followed immediately by marked muscle twitching, which passed away in three minutes. The injection was then repeated with the same effect. One cubic centimeter was then given. The symptoms were severe and continued fifteen minutes. Then 1.25 cc. were injected. Twitching and convulsive movements became very marked and continued so for a few minutes, then disappeared slowly and were entirely absent in thirty minutes. Recovery was rapid and, within an hour of the last injection, the dog was quite normal and ate eagerly. The total amount injected was 3.75 cc. containing 1.965 gram of meta-cresol acetate. This was equivalent to 1.415 gram of meta-cresol or 0.202 gram per kilo. Gibbs and Hare⁸ found that 0.15 gram of meta-cresol per kilo., injected intravenously at one time, was fatal.

Intraperitoneal injection. The dog used in the experiment just described was kept under observation for four days and in all respects behaved like a normal dog. On the fifth day 4.2 grams of meta-cresol acetate in 20 cc. of olive oil were injected into the peritoneal cavity. Muscle twitching soon followed but ceased in four hours. The next day the dog was quite apathetic and ate very little. On the following morning he was dead. At autopsy a mesenteric hemorrhage was found opposite the site of injection. Otherwise everything was apparently normal.

Local action. The local action of meta-cresol acetate was next studied. A dog had two feet bound with cotton compresses saturated with the acetate. The compresses were removed after

⁷On account of the great danger of producing oil embolism care was taken to introduce slowly only very small doses of the oily liquid at each injection.

⁸Gibbs and Hare: *Archiv. f. Phys., Suppl.* Band., 1889, p. 271.

twelve hours and the feet were found to be unaffected. There was no inflammation whatever. Two days later, under cocain anesthesia, two longitudinal incisions, about two inches long, were made in the skin of the back of the neck. On the following day one of the wounds was covered with a cotton compress saturated with meta-cresol acetate, and the other with dry absorbent cotton. This treatment of the wounds was repeated daily for three days. As it was very difficult to keep the compresses in position their use was then discontinued and the treated wound was painted with meta-cresol acetate three or four times a day on four successive days. At no time did the dog appear to be annoyed by the acetate and the two wounds healed with equal rapidity.

Subcutaneous injection. Subcutaneous injections of meta-cresol acetate were very well borne by dogs. In one animal, weighing 7.5 kilos, the injection of 3.5 grams of undiluted meta-cresol acetate, and also, later, of 5 cc. of olive oil solution containing 3.14 grams of the acetate, was followed by only slight transient local reaction. Another dog, weighing 8.5 kilos, received 6.29 grams of meta-cresol acetate. The swelling that followed did not disappear but softened and, after puncture three days later, 100 cc. of bloody pus were removed. The skin about the point of puncture was very thin and an open wound soon resulted, but the dog kept this clean and it healed rapidly.

Excretion and effects on metabolism. Jonescu⁹ found that of 4 grams of meta-cresol, given in four daily doses to a dog weighing 11.96 kilos, none was excreted in the feces and only 1.768 grams or 46.5 per cent in the urine. In another experiment in which 1 gram of meta-cresol was given on each of three successive days, the same dog excreted in the urine 1.505 gram, or 50.17 per cent of the amount administered. Similar results were obtained in this work with meta-cresol acetate.

A dog weighing 4.19 kilos was kept upon a daily diet of 75 grams of hashed meat (prepared and preserved as described by Gies¹⁰), 20 grams of cracker meal, 15 grams of lard, 5 grams of

⁹Jonescu: Biochem. Zeit., 1906, i, p. 399.

¹⁰Gies: Am. Journ. Physiology, 1905, xv, p. 235; Proc. Soc. Exp. Biol. and Med., 1908, v, p. 27.

bone ash and 175 cc. of water. The dog was fed at 9 a.m., but a little of the meat was reserved until noon when it was given with an *empty* gelatin capsule (control). After the dog had been kept on the above diet for eight days the collection of urine and feces was begun. The excreta for four days were obtained. On the fifth day a gelatin capsule containing 0.512 gram of meta-cresol was given two hours after feeding. Nothing unusual was noticed within the next ninety minutes, but on the following morning the urine was found to be contaminated with vomit. This day's urine was discarded. During the next four days the dog received 2.0488 grams of meta-cresol acetate, equivalent to 1.475 grams of meta-cresol, in four approximately equal daily doses, which were given in gelatin capsules in meat three hours after feeding.

The feces of this period were treated with boiling H_2SO_4 solution and the volatile matter distilled from the mixture. No phenols could be detected in the distillate. The urines of each period were combined and analyzed. The analytic methods which were employed are indicated in the following summary.

Total nitrogen: Kjeldahl method.

Urea: Benedict method.¹¹

Total sulfur: Benedict method.¹²

Total and inorganic sulfates: Folin methods.¹³

Glycuronic acid: Distillation with HCl solution and precipitation with phloroglucin, according to Tollens.¹⁴

Phenols: Distillation as in Kossler and Penny's method,¹⁵ but, as the action of iodine solutions upon meta-cresol has not yet been studied, a bromination process was used. Ditz and Cedivoda,¹⁶ and others, have studied the action of bromine water upon solutions of meta-cresol and have found that a tri-brom derivative is readily formed. In our experiments Lloyd's¹⁷ modification of the Koppesschaar method was used. It

¹¹Benedict: J. Biol. Chem., 1910, viii, p. 405.

¹²Benedict: J. Biol. Chem., 1909, vi, p. 363.

¹³Folin: J. Biol. Chem., 1906, i, p. 131.

¹⁴Tollens: Z. Phys. Chem., 1909, lxi, p. 95; lxiv, p. 39.

¹⁵Kossler and Penny: Z. Phys. Chem., 1893, xvii, p. 117.

¹⁶Ditz and Cedivoda: Z. f. Angew. Chem., 1899, pp. 873 and 897.

¹⁷Lloyd: J. A. Chem. Soc., 1905, xxvii, p. 16.

was first tried with solutions of pure meta-cresol and found to give accurate results.

TABLE IV
Analytical Results. Metabolism Experiment on a dog

PERIODS	DOG	URINE							ANALYTIC RATIOS		
		Total N	Ammonia and Urea N	Total Sulfur	Total Sulfate Sulfur	Inorganic Sulfate Sulfur	Glycuronic acid Calculated as the Lactone*	Phenols Calculated as Meta-Cresol	Total Nitrogen Percentage of Ammonia and Urea Nitrogen	Total Sulfur Percentage of Total Sulfate-Sulfur	Total Sulfate Percentage of Inorganic Sulfate
Four Days Each	Average Weight	grams	grams	gram	gram	gram	gram	gram	per cent	per cent	per cent
Fore period. . . .	4.2	12.5	11.24	0.685	0.492	0.462	0.247	0.014	89.91	71.83	93.78
Meta-cresol acetate period. .	4.2	11.95	10.23	0.629	0.479	0.206	1.175	0.674	85.61	76.12	43.00

*It may be noted that in these urines, Tollens' colorimetric method gave results indicating a glycuronic acid content of at least 6.25 and 7.75 grams respectively. A number of normal dog urines were found to give the reaction in correspondingly high dilution. Apparently, then, the test may be more delicate than Tollens supposed or, as is much more probable, some dog urines contain a substance which either is not precipitated by basic lead acetate and ammonium hydroxid or does not yield furol on boiling with 12 per cent HCl solution, but which does give Tollens' test for glycuronic acid.

If the amount of cresol excreted in the first period is subtracted from that eliminated in the second period the difference is 0.66 gram or 44.74 per cent of that given. The ethereal sulfate excretion for the second period was equivalent to 273 mg. of sulfur, while in the control period it was only 30 mg. The difference, 243 mg., if present as meta-cresol sulfuric acid, would be equivalent to 0.816 gram of meta-cresol. Similarly with the glycuronic acid, the difference in the excretion for the two periods is 0.928 gram (calculated as the lactone), corresponding to 0.569 gram of cresol. The total amount of cresol accounted for by the increased ethereal sulfate and glycuronic acid excretion is 1.385 gram or 93.91 per cent of the amount taken. Apparently, then, somewhat more than half of the meta-cresol derived from the meta-cresol acetate was oxidized, though without any considerable decomposition of the aromatic nucleus; and these oxida-

tion products, as well as unchanged meta-cresol, were excreted mainly as salts of the conjugated sulfuric and glycuronic acids.

Oxidation products of meta-cresol acetate in the urine. Very early in the course of this work it was noted that the urine of dogs that received meta-cresol acetate became very dark on standing, thus indicating the presence of homologues of pyrocatechol and hydroquinon. Preusse¹⁸ having reported that he could find no meta-oxybenzoic acid or di-hydroxy-toluenes in the urine of a dog to which he administered 10 grams of meta-cresol, it was thought advisable to attempt the isolation of such oxidation products as might theoretically be expected. These were meta-oxybenzoic acid, homo-pyrocatechol, iso-homo-pyrocatechol and hydrotoluquinon. Accordingly, the combined urines of four dogs that had received a total of 50.7 grams of meta-cresol acetate by mouth, and 22.7 grams subcutaneously, were evaporated in several portions to syrups, and the latter extracted with alcohol. The combined extracts were evaporated to a syrup and allowed to crystallize. The crystals were used in an unsuccessful attempt to isolate the potassium salt of meta-cresol sulfuric acid; nothing but urea was obtained. Some of the filtrates from the crystals were placed in a vacuum desiccator over concentrated sulfuric acid. After standing several days a few crystals formed in the thick syrup obtained, but they could not be satisfactorily separated from it.

The combined filtrates were strongly acidified with sulfuric acid and continuously distilled in a current of steam. At the end of a period of thirty hours considerable cresol was found to be passing into the distillate. The distillation was interrupted and the liquid in the distillation flask, after cooling, was thoroughly extracted with ether in a continuous extraction apparatus. The ether extract was shaken with sodium bicarbonate solution. The latter was subsequently washed with a little ether. The combined ethereal solutions were distilled until all the ether was removed. A current of steam was then passed through the residual liquid until the distillate no longer gave a precipitate with

¹⁸Preusse: Z. f. Phys. Chem., 1881, v, p. 57.

bromine water. The liquid remaining in the distilling flask was filtered from a tarry residue, evaporated to a syrup and set aside in a refrigerator. No crystals appeared even after several weeks. The syrup was finally dissolved in water and lead acetate added to complete precipitation. Basic lead acetate was added to the filtrate; a slight precipitate was produced.

The first lead acetate precipitate was decomposed with sulfuric acid. The liquid was filtered and the filtrate extracted with ether. This extract, after distillation of the ether, left a red oil which could not be made to crystallize. Ferric chloride when added to the aqueous solution of the oil produced a green color which soon changed to brown. When ammonium hydroxid solution was added after treatment with ferric chloride, the color changed to violet. With sodium carbonate solution the color changed to a red violet. The substance reduced ammoniacal silver solution in the cold and Fehling solution on slight warming. Ammonium hydroxide turned the aqueous solution of the substance green. A boiling solution in chloroform gave a blue-green color on treatment with solid potassium hydroxide.

The precipitate produced by *basic* lead acetate yielded a smaller quantity of a substance having apparently the same properties as that obtained from the first precipitate. Exactly the same reactions were given by a sample of homopyrocatechol, which was, however, not quite pure.

The filtrate from the lead precipitates was freed from lead with hydrogen sulfid, filtered and evaporated. The residue was a brown syrup which gave no color with ferric chlorid nor with chloroform and potassium hydroxid. When treated with anilin and acetic acid the liquid remained clear. Apparently it was neither toluquinon nor hydrotoluquinon.

The sodium bicarbonate extract was acidified with sulfuric acid and extracted with ether. The ether was distilled off; it left a brown crystalline residue. This was pressed on a porous plate. It was then dissolved in water and treated with animal charcoal, but with little effect. The liquid was evaporated to crystallization. The crystals were dried on a porous plate and dissolved in boiling toluol. On cooling, crystals were deposited.

These consisted of small needles and irregular hexagonal plates which melted at 190 degrees (uncorrected). A solution of some of the material gave a very faint green coloration with ferric chlorid solution. A few crystals, dissolved in concentrated sulfuric acid solution and warmed, gave an orange-red liquid. The crystals tasted sweet. When 63.3 mg. were dissolved in water and titrated with $\frac{N}{10}$ ammonium hydroxid solution, using Congo red as the indicator, 4.3 cc. of the alkalin solution were required. Calculated for $C_6H_4(OH)COOH$, the volume is 4.6 cc. After its titration, the liquid was acidified with sulfuric acid and extracted with ether. The ether was allowed to evaporate spontaneously in an open dish. It left a yellow ring with pure white crystals in the center. These gave no color with ferric chlorid solution and melted at 197° (uncorrected). Probably the substance was meta-oxy-benzoic acid, which possesses the above properties and melts at 200°.

Unfortunately this work was begun several weeks after the collection of the urines and was frequently interrupted by other work, so that whatever hydroxy-toluenes were originally present may have been, at least in part, oxidized and condensed to unknown substances. This was indicated by the very dark color of the urines under examination.

Attempts were made to isolate the conjugate glycuronic acid by Külz's method¹⁹ but without success. In one case, urine from an 8.1 kilo dog that had received 8.8 grams of meta-cresol acetate was used immediately after collection. The precipitate produced by basic lead acetate in the aqueous solution of the alcohol-ether extract was decomposed with hydrogen sulfid and the filtrate from the lead sulfid was evaporated. The residue was a non-crystallizable syrup. It was hydrolyzed with hydrochloric acid solution. The liquid gave a very intense reaction for glycuronic acid with naphthoresorcin but, after neutralization, gave no color with ferric chlorid solution. On warming, however, an odor resembling that of quinon was noted. The rest of the liquid was extracted with ether and this evaporated. The resi-

¹⁹ Külz: Z. f. Biologie, 1890, xxvii, p. 247.

due gave a negative reaction with ferric chlorid solution. When dissolved in chloroform and boiled with potassium hydroxid solution a red-brown color was obtained. Toluquinon gives the same reaction. Possibly this substance was present.

To the filtrate from the basic lead acetate precipitate, sulfuric acid solution was added in a quantity sufficient to effect complete precipitation. The precipitate was filtered out and washed. The filtrate was extracted with ether, the extract evaporated and the oily residue dissolved in water. It gave a slight transient green coloration with ferric chlorid solution, but no other color appeared after the addition of ammonium hydroxide and sodium carbonate solutions. On warming the liquid with ferric chloride solution, a quinon-like odor was noticed. Ammoniacal silver solution was reduced in the cold and Fehling solution on warming. The remainder of the liquid was evaporated to dryness. The amorphous residue was dissolved in chloroform and this solution boiled with solid potassium hydroxid. A reddish brown color was produced. It seems quite probable that hydrotoluquinon was present.

In a number of cases it was found that, after administering meta-cresol acetate by mouth or subcutaneously, the urine did not decompose. Such urines were kept from forty-eight to fifty-three days without becoming alkaline. In the dogs that received meta-cresol acetate by mouth this was the case only when the dose was just about large enough to cause muscle twitching. Urines voided after subcutaneous injections which caused no general symptoms were preserved without the addition of an antiseptic for forty days. The minimum amount that was found to do this was 4.1 grams for a dog weighing 8.5 kilos. The next day the same dog received 6.3 grams. The urines of the following three days, separately collected, remained acid for forty days.

III. SUMMARY OF GENERAL CONCLUSIONS

1. Meta-cresol acetate, a colorless liquid of pleasant odor, is practically insoluble in water, is not readily hydrolyzed and is a strong antiseptic agent.

2. It does not exhibit the marked protein-coagulating action of free meta-cresol.

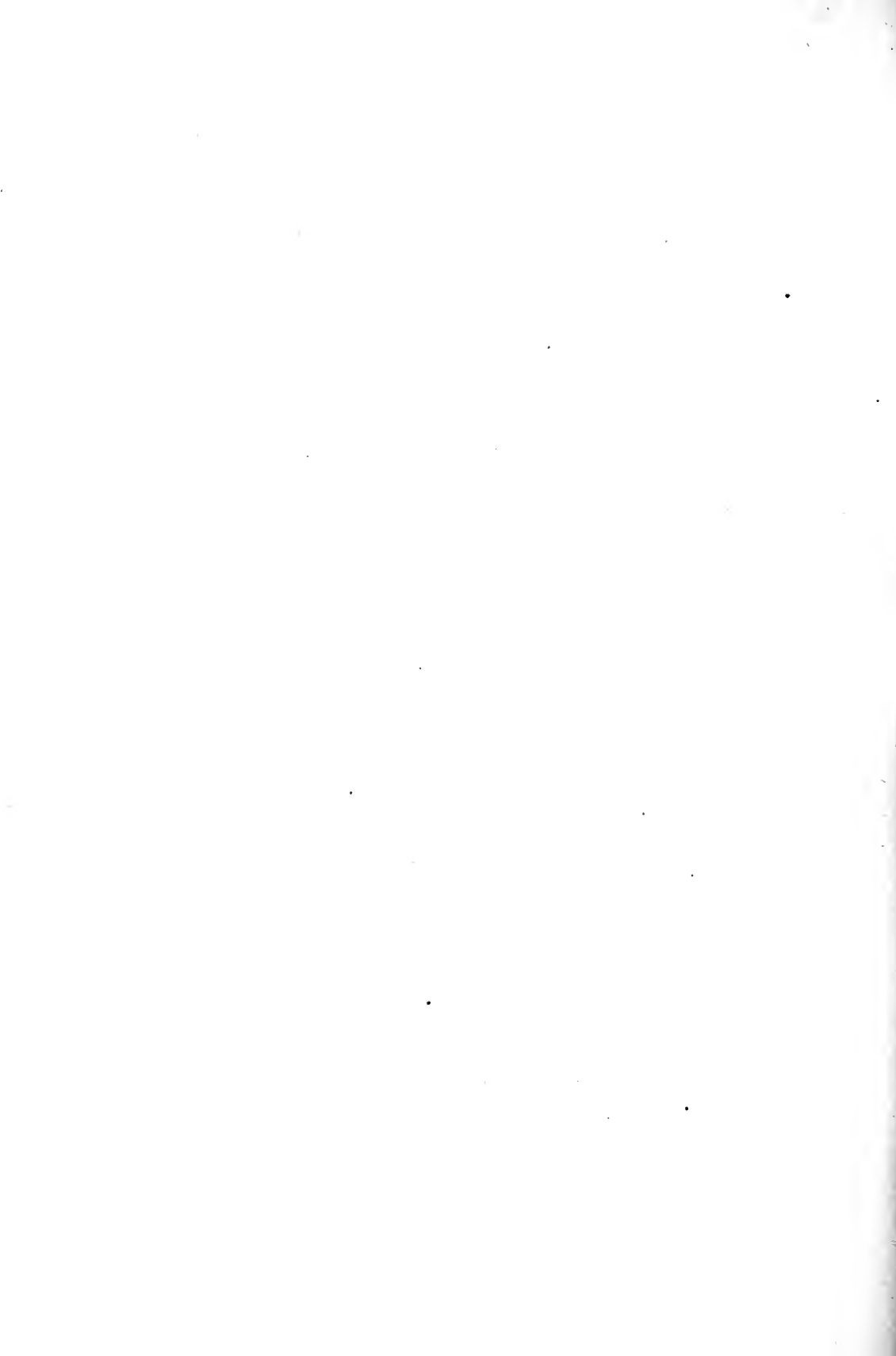
3. It is probably unaffected in the stomach but is decomposed in the intestine.

4. It is non-corrosive and has very little harmful local effect except in the intestine.

5. When injected into the dorsal lymph sac of frogs or given by mouth to dogs, it is about as poisonous as the equivalent amount of meta-cresol.

6. When taken into the body it is partially oxidized to dihydroxy-toluenes and meta-oxy-benzoic acid, which are in great part excreted as the conjugate sulfuric and glycuronic acids.

It gives me great pleasure to here thank Prof. William J. Gies for suggesting this investigation and for his interest and advice during the course of the work.



ON THE ACTION OF SENECHIO ALKALOIDS AND THE CAUSATION OF THE HEPATIC CIRRHOSIS OF CATTLE (PICTOU, MOLTENO, OR WINTON DISEASE)

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In a number of the colonies a disease affecting horses and cattle and inducing hepatic cirrhosis has been known for some years. In New Zealand, in which it occurs chiefly but not exclusively in Southland, it is known as Winton disease, in South Africa as Molteno disease, and in Canada as Pictou disease, from its occurrence in the neighborhood of Pictou, Nova Scotia, exclusively. The symptoms of the disease are described as practically identical in the three localities. Cattle¹ are observed to be "unthrifty" for some time, but definite symptoms appear only three or four days before death, and commence often in diarrhoea, diminished milk, dry and staring coat and disinclination to feed. The diarrhoea is not necessarily severe, but is often followed by straining which increases in intensity and frequency and may lead to eversion of the rectum and rupture of its vessels. Considerable pain appears to be felt, the cattle groaning and lying down or becoming frenzied and charging anyone who approaches. Eventually unconsciousness sets in and death follows in two to four days after the first definite symptoms were observed.

The liver is found in some cases to present the appearance of chronic cirrhosis and feels leathery and tough when cut. In others the only change in this organ is marked venous congestion. It is generally of small size and blue-slate in color and the edges are rounder than usual. The gall-bladder is distended

¹ W. H. Chase: *Agricultural Journal*, Cape of Good Hope, xxv, p. 675, 1904.

with very viscid yellowish black or black bile, and the interior shows a number of red spots of the size of a pin's head. The urinary bladder and heart may also show these petechiæ. The first three stomachs present nothing abnormal, but the fourth has red hæmorrhagic spots. The folds are thickened by the submucous exudation of gelatinous fluid. The intestine is inflamed around the openings of the bile ducts and the lower bowel may be congested from straining.

The disease is of great economic importance, for in Nova Scotia it is calculated it has been the cause of the loss of several thousand head of stock, and in the East London district of South Africa, Dixon² states that it has rendered horse-breeding impossible as the mares contract the disease after two years or more grazing. A number of investigations have been carried out as to its causation more especially in Canada where its limitation to the district immediately around the town of Pictou has been especially remarked. It seems to be generally believed in this neighborhood that the disease is of recent origin and it has been associated popularly with the introduction of *Senecio jacobaea* or ragwort (Stinking Willie) in the ballast of a ship from Scotland about fifty years ago. The disease certainly rose a few years afterwards and occurred more particularly on the farms on which the plant was prevalent. But investigations carried out in 1882 under government auspices failed to connect the disease with the weed, and cirrhosis of the liver was therefore put on the list of contagious diseases, animals affected with it were slaughtered and the buildings in which they had been kept were disinfected.

The pathology of the disease was studied by Osler, Wyatt Johnston and especially exhaustively by Adami,³ who states that the main lesion is an extreme condition of cirrhosis of the liver, "the fibrous tissue not only being along the vessels between the lobules, but extending in between the individual cells, the organ being enlarged and having a smooth or more rarely a finely granular surface." In the animals examined by him in Canada there

² Report of the Director of Agriculture of the Cape of Good Hope, 1906, p. 41

³ Montreal Medical Journal, February, 1902.

was abundant production of thin bile and the gall-bladder was generally very full and the fæces well stained. The abdominal lymph glands were generally large and succulent and moderate ascites was present, with gelatinous œdema of the mesenteries and walls of the intestines. Numerous follicular ulcers were found in the fourth or true stomach but were generally in a cicatrized condition except in very acute cases. In animals killed in early stages, the most noticeable features are fatty degeneration of the liver cells with great congestion of the liver vessels (Johnston). This stage appears to be succeeded by rapid destruction of the liver cells and replacement of these by delicate new connective tissue. Johnston and Adami isolated an organism from the organs, but were unable to induce cirrhosis in animals inoculated by it and it subsequently proved to be the colon bacillus. Johnston and Hammond showed that the blood of cattle affected with the disease agglutinated the organism isolated from the liver, which apparently confirmed their view that this organism is the cause of the disease. The cogency of this argument has been lessened however by the observations of Ford⁴ that in over 80 per cent of the livers of normal animals bacteria may be found. Adami (1902) still appears to hold that there is a direct relationship between these organisms and the cirrhotic process, but considers that they are not the primary cause of the disease, but that, gaining entrance through a gastric ulcer or other inflammatory condition they induce the characteristic changes in the liver.

There has always been a popular impression in Pictou that the disease was associated with the ragwort, but the earlier experiments (1882) seemed to negative this idea. In 1902, Gilruth⁵ was led to examine the effects of the *Senecio jacobaea* of New Zealand by the observation that cattle on a station affected by Winton disease had been eating this plant. He fed two healthy calves with a ration containing this weed, and found that they

⁴ Journ. of Hygiene, i, p. 277, 1901.

⁵ Tenth Report of the Department of Agriculture, Wellington, New Zealand, p. 300.

became ill after about eighteen days and died on the twenty-eighth and thirtieth day of the experiment. The symptoms were typical of Winton disease. Post mortem the peritoneal cavity contained straw colored fluid and the gall-bladder was full of dark fluid of the consistency of treacle. The connective tissue of the liver was much increased especially around the portal canal and smaller interlobular branches of the portal vein; from these bands of finer fibres extended between the liver cells sometimes isolating them completely. Areas of congested portal capillaries were found irregularly in the sections. The liver cells were distorted in shape and atrophied. The capsule of the liver was thickened and trabeculae passed from it into the tissue.

In South Africa, Chase⁶ found that the inoculation of the bile, blood and stomach contents of an animal dead of Molteno disease had no effect upon healthy cattle. After Gilruth's results in New Zealand appeared, the *Senecio burchellii* of South Africa fell under suspicion and Chase found that about 4 oz. of this plant given on four successive days to an ox caused his death with the usual symptoms and the typical post mortem appearances on the fifth day. He does not state whether the plant was given green or dry.

Dixon notes (1906) that the *Senecio latifolius* grows abundantly in the area in which the hepatic cirrhosis is observed in the East London district, South Africa.

In Canada Pethick⁷ carried out an admirably planned investigation of the subject in 1903; sixteen head of cattle were kept in a new stable and fed on hay from Pictou, while sixteen controls, kept in an old stable in which several cattle had previously died of the disease, received hay from Quebec, where the disease is unknown. These sixteen controls remained healthy for twenty-three months, though they were placed in contact with the diseased animals and in some cases were inoculated with the blood or ascitic fluid of animals that had died of the disease; when

⁶ loc. cit.

⁷ Department of Agriculture, Canada. Health of Animals: Special Report on Pictou Cattle Disease, Ottawa, 1906.

slaughtered at the end of twenty-three months their organs were free from disease. Of the sixteen cattle to which hay from Pictou was supplied, fifteen died of the disease as was verified by the post mortem examination, and the sixteenth animal was found to present the characteristic lesions of the disease when it was slaughtered at the end of twenty-six months. One animal fed with chopped ragwort mixed in Quebec hay died in twelve months of acute cirrhosis, while the control fed on Quebec hay remained healthy. It was found that sheep can be fed on ragwort with comparative impunity, though they also suffer after some time and the flesh finally assumes a yellowish tint (jaundice?). Sheep may thus be employed to eat down the ragwort on farms infected with the weed and thus to extirpate it. Rutherford⁸ states that sheep eat ragwort with impunity whether the plant is in the green or in the dry state. Cattle refuse to eat it in the green state and the poisoning seen in Canada arises only from the dried ragwort in the hay. But cattle in South Africa and New Zealand eat the green plant also when other fodder is scarce. (Chase.)

The experiments of Gilruth, Chase and Pethick show beyond doubt that poisoning with different species of Senecio is the cause of the disease known as Pictou disease in Canada, Molteno disease in South Africa and Winton disease in New Zealand. The species of Senecio hitherto incriminated are *S. jacobæa* in New Zealand and Canada, and *S. burchellii* and probably *S. latifolius* in South Africa, but it is possible that other species may be equally poisonous. Curiously enough the *S. jacobæa* which grows in abundance all over the eastern districts of England and Scotland (from which it was introduced into Canada) is not known to cause any symptoms in cattle, and the *S. vulgaris* or groundsel which is found equally widely distributed is also reputed to be harmless.

As regards the view that the hepatic cirrhosis is a bacterial disease, the experiments given above indicate that the rôle played by the microbes is a secondary one, which bears the same relation to the disease as the terminal infection occurring in other chronic wasting diseases.

⁸ Report of the Veterinary Director General and Live Stock Commissioner, Ottawa, 1909, p. 21.

The chemistry of the *Senecio* genus has not received much attention. Grandval and Sejour⁹ found in the common groundsel (*S. vulgaris*) two alkaloids, senecionine ($C_{18}H_{25}O_6N$) and senecine, but make no statement as to their toxicity. Dr. H. E. Watt¹⁰ has recently examined the *S. latifolius* of Cape Colony, which is believed to be responsible for at any rate some of the outbreaks of disease in South Africa, and has succeeded in isolating from it two alkaloids, which in the crude state amounted to 1.72 per cent of the dried plant gathered before flowering and 0.76 per cent in those gathered after flowering. One of these *senecifoline* corresponded to the formula $C_{18}H_{27}O_8N$, forms crystalline salts of which the nitrate melts and decomposes at 240, the hydrochloride at 260, and the aurichloride at 220; it can be decomposed into an acid and base by alkali and both of these products were isolated and examined. The second alkaloid, *senecifolidine*, $C_{18}H_{25}O_7N$, also forms crystalline salts of which the nitrate melts at 145.

Professor Wyndham R. Dunstan, under whose direction this chemical investigation was carried out kindly sent me the nitrates of these bases for pharmacological examination.

SENECIFOLINE NITRATE

Injected into frogs in quantities of 10 to 40 mgs. senecifoline induced no symptoms whatever for five to fifteen days, and the quantity given seemed to exercise no influence on the interval between the injection and the onset of symptoms. At the end of this time the condition varied in different animals. In some the position was fairly normal, but the respiration had ceased and gaping movements were made apparently in attempts to vomit, for sometimes the stomach was protruded through the mouth. In others clear blood escaped from the mouth and the animal showed all the symptoms of collapse from hæmorrhage. In others strychnine-like spasms and muscular twitching were

⁹ Compt. rend. de l'acad. des Sciences, Paris, 120, p. 1120, 1895

¹⁰ Trans. Chem. Soc., 95, p. 466, 1909.

present. Death followed in a few hours after the first symptoms developed. The appearances post mortem varied greatly in different frogs. In some the abdominal cavity was found to contain quantities of clear fluid; the stomach was often found to be normal or somewhat congested, but hæmorrhage had occurred in the bowel which sometimes contained much blood-stained mucus; the cloaca and lower bowel often were filled with a mixture of blood and mucus. Hæmorrhage from the lungs was the chief abnormal feature in several frogs.

A number of experiments were performed on cats, white rats and rabbits, and the reaction of these animals to the alkaloid was uniform in most particulars. The drug was generally injected hypodermically but the same results were obtained when it was given by means of the stomach tube in cats.

In the cat senecifoline nitrate induces two sets of symptoms which are quite distinct in character.

Acute symptoms. After a small dose, *e.g.*, 0.01 to 0.02 gm. per kg. has been injected hypodermically there is often some salivation which continues for half an hour or longer. After large quantities, *e.g.*, 50 mgs. per kg. this feature is more marked, the saliva pouring from the mouth and later falling in long strings from the jaws. The pupils may be somewhat dilated, but these symptoms disappear in the course of twenty-four hours and apart from one or two stools of rather loose consistency the animal appears to have recovered completely until it shows the symptoms described later. In somewhat larger doses vomiting is often induced in the course of the first two hours. When quantities of 0.1 to 0.2 gm. per kg. are injected into cats, the profuse salivation is accompanied by vomiting, the pupils are widely dilated, the respiration is extremely accelerated (up to 300 or more per minute). The animal shows the sudden shrinking movements, which are often seen under cocaine and which have been construed as indicating hallucinations, and somewhat later violent clonic convulsions may be developed. These resemble closely those developed under cocaine and other convulsant poisons, periods of very active movement alternating with pauses during which the animal lay still and in which the respiration was

slow at first but gradually accelerated until it culminated in a renewed seizure. All of these symptoms pass off in the course of two hours or less after the injection and next day the animal appears fairly normal; the appetite may seem poor or some depression may be present but very often no further symptoms can be made out for several days.

In the rabbit and rat I have not been able to elicit any primary symptoms of so marked a character. In the rat even 1.0 gm. per kg. caused no convulsions but there did seem to be some excitement, the animal running about more, climbing up its cage and making gnawing movements. The respiration was also accelerated at first, but it was difficult to determine how far this was due to the injection as such and how far to the specific action of the drug. Smaller doses of senecifoline nitrate were followed by no symptoms whatever for many hours after the injection and then gave rise to the characteristic changes which will be described below.

These acute symptoms point to an action of senecifoline on the central nervous system similar to those observed under a number of other convulsant poisons. The salivation in cats is the most common feature and appears to be merely an accompaniment of the nausea which under larger doses causes vomiting. The acceleration of the respiration resembles that seen under cocaine and the convulsions and apparent hallucinations are also commonly elicited by this drug and its congeners. The acute symptoms are thus referable to stimulation of the upper part of the central nervous axis similar to that induced by cocaine and apomorphine. No distinct reflex in the spinal reflex irritability could be made out, so that the action is confined to the medulla oblongata and higher centres.

In one cat anaesthetized with paraldehyde a blood pressure experiment was performed and the salivary secretion was determined at the same time by a cannula inserted in the submaxillary duct. The intravenous injection of small quantities 0.01 to 0.025 gm. had little or no effect on the blood pressure or rate of the heart. Larger amounts, 0.05 to 0.15 gm. induced a rapid fall in blood pressure followed by a return to the normal, which was

reached in two to five minutes. The salivary secretion was unaffected throughout and the pupil remained unchanged in size.

The local application to the eye of the cat of senecifoline nitrate in 5 per cent solution caused neither irritation nor anaesthesia and had no effect on the size of the pupil. The dilatation of the pupil observed among the acute symptoms is therefore to be ascribed to the general excitement as in the case of many other excitants.

The acute symptoms seem to arise from a stimulant action on the upper part of the central nervous axis. As in the case of many other poisons acting in this way the symptoms are much more readily elicited in the cat than in the rodents.

Subacute Symptoms. After the recovery from the primary symptoms cats remained to all appearance quite normal for at least twenty-four hours and often for three days up to a week. After small doses, *e.g.*, 0.07 gm. per kg., salivation was generally absent and no symptoms developed at all for several days. In those remaining without symptoms for the longer periods, some loss of weight was generally noted, but this was often insignificant until the more severe subacute symptoms set in. These were often introduced by a stool of rather loose consistency, and by loss of appetite, the animal eating nothing or often vomiting what little it had swallowed. Weakness and disinclination to move soon appeared, and somewhat later when the animal was liberated from its cage and encouraged to walk, the gait was unsteady and staggering; the legs were sometimes curiously stiff and wide apart. When sitting still the body swayed to and fro, the head fell lower than normally and though it was raised when the animal was disturbed it assumed the same position very soon. This condition of apathy soon deepened into stupor in which the animal lay still and could be aroused with difficulty or somewhat later failed to respond to sounds or touch. The respiration became gradually slower, the temperature fell 4 to 5 below normal, the pulse was also slow. Finally the respiration gradually ceased, the heart continuing to beat for some time afterwards and few or no convulsive movements occurring at death. In one experiment on the cat a large stool consisting of almost

unaltered blood was passed before death and at the autopsy blood was found to have exuded from the rectal mucous membrane in large quantity. The urine examined during these symptoms sometimes contained a trace of protein, but was generally free from it.

These symptoms succeeded each other rapidly, death occurring about twenty-four to forty-eight hours after their first appearance in the majority of cases. Thus an animal often remained apparently normal for four to five days after the drug was administered but died on the day after symptoms first set in. In some experiments in which a large dose was injected, death occurred in two days, and in these the weight was found to have fallen about 10 per cent, while in animals surviving four to five days, as much as 20 per cent of the original weight had been lost. Even in animals which recovered after senecifoline injection some loss of weight was often noted.

The smallest dose which proved fatal to the cat was 0.016 gm. per kg. As a general rule the dose used in the cat was about 50 mgs. per kg., and this amount proved fatal in four to six days whether it was injected hypodermically or given by the stomach tube.

In the rat the symptoms were very similar, setting in with loss of appetite (but with no diarrhoeic stool), after which increasing weakness and deepening stupor and coma followed; the respiration became slow and finally ceased, the heart beating for some minutes longer. The senecifoline nitrate was always injected hypodermically, and great variation was found in the quantity which proved fatal in different animals. Thus in one rat 0.044 gm. per kg. proved fatal in three days, while others survived 0.135, 0.16 and even 0.22 gm. per kg. The interval between the injection and death again showed great variation, some rats dying within twenty-four hours of the injection, while others survived five to six days. The amount of alkaloid injected did not seem to bear any definite relation to the time of survival. In rabbits the symptoms resembled those in cats, and the fatal dose was not ascertained in them.

The post mortem appearances differed considerably in different animals of the same species even when they had received the same amount of the poison and had survived the same time. In the cat there was generally an unusual amount of fluid in the abdominal cavity and this was sometimes of a bright yellow color. In one cat in which there was marked jaundice the abdominal cavity was filled with fluid of a deep bile color. Some congestion of the great omentum was often present and this was sometimes very marked; small ecchymoses were present in the omentum and fat deposits in one or two animals. The stomach generally contained black masses of half digested blood especially towards the pyloric orifice, and the duodenum also contained some effused blood mixed with mucus, or sometimes was filled with black masses of blood; congestion of the stomach and upper part of the intestine was generally to be made out, but sometimes there was no abnormal appearance in the stomach or bowel except some slight congestion of the vessels.

The liver was swollen and congested and bled freely when cut into. A section presented areas of dark and light color. The gall bladder was distended with very dark colored viscous bile which could only be expressed from it with difficulty. The kidneys sometimes presented the appearance of fatty degeneration. Small hæmorrhages were often found in various organs, such as the omentum, lungs, pancreas, and were present in most cases in the stomach and intestine. In the rabbit, similar lesions were found at the autopsy. In one case a quantity of brownish fluid was found in the pleural and pericardial cavities as well as in the abdomen.

In the rats hæmorrhages into the stomach and duodenum were almost invariably found and the contents of the upper part of the gut were thick mucus, stained pink with blood. The gastric hæmorrhage was confined to the pyloric half of the organ or true stomach. In the peritoneal cavity bile stained or blood stained fluid was often present, sometimes in large amount, and in several instances fluid was present also in the pleural and pericardial cavities. The tissues were rarely jaundiced. The liver was generally enlarged and soft and contained large areas of

intense congestion. Hæmorrhages occurred in a number of organs. The kidneys generally appeared normal. In the rat as in the cat, the thoracic organs were generally normal in appearance, save for occasional discrete hæmorrhages in the lungs and rare pleural effusions.

Dr. C. Bolton kindly examined some of the organs microscopically for me, and found marked congestion and hæmorrhages in the liver; the hæmorrhage in some cases was confined to the peripheral half of the lobules, which were mapped out by it, in other instances both hepatic and portal veins seemed to be equally involved. When the peripheral part of the lobule alone was affected, the hepatic cells in the centre were normal in appearance, but further outwards they became distorted by the blood cells and stained badly and towards the interlobular vein they were quite colorless and evidently in process of disintegration. Large areas containing many lobules were necrosed, the liver cells not taking the stain at all, and here hæmorrhages were also present though not in greater degree than in those parts in which the hepatic cells were in part preserved, so that this necrotic action appeared to be independent of the hæmorrhages. In acute poisoning the liver cells often contained globules of fat, but this did not seem to be in excess of that seen in normal animals. In less acute cases this fat was not present. There was considerable infiltration of round cells round the portal canal, especially involving the smaller bile ducts and often occupying their lumen and also extending outwards from the ducts between the liver cells; and this was present in subacute cases though it was more marked in chronic poisoning. Catarrh of the smaller bile ducts was indicated by shedding of epithelium.

The liver symptoms suggested that senecifoline might have some of the properties of toluylendiamine, and its effect on the blood was therefore examined. I may mention that the blood clotted normally in animals poisoned with the alkaloid, except when marked jaundice was present and the peritoneal fluid also clotted after standing some time. There was no hæmolysis in the blood drawn from the heart before or after death in animals poisoned with senecifoline, and no hæmoglobin appeared in the

urine. Normal cat's blood diluted with 9 parts of saline solution was put in the incubator at 38 with a varying proportion of senecifoline nitrate. After sixteen hours a very slight degree of lysis had occurred in the tubes containing senecifoline, while the controls remained unchanged. In the senecifoline tubes there was also some methæmoglobin and this later passed into dark amorphous insoluble masses. This lysis and methæmoglobin formation was fairly obvious in tubes which contained 1 of senecifoline nitrate in 300 and was barely visible when the concentration was 1:1400. Strychnine nitrate added to control tubes and exposed in the same way had no effect on the blood pigment and produced no lysis. So that this action of senecifoline cannot be attributed either to the nitrate half of the molecule or to the weakness of the base inducing an excess of H ions. Senecifoline thus has a weak hæmolytic action and leads to methæmoglobin formation in the shed blood, thus resembling some of the oxidizing inorganic salts. The concentration necessary to elicit this is much higher than that reached in the living tissues and in the intact animal the blood was not found to be lysed nor to contain methæmoglobin, so that the subject was not pursued further. It may be mentioned however that some of the oxidizing poisons, such as the iodates, induce similar lesions in the rat's stomach to those described under senecifoline.

Chronic poisoning was induced in two experiments in order to compare the symptoms elicited with those observed in cattle in Pictou disease.

A young cat of 1200 gms. weight.

- 5. II. 30 mgs. senecifoline nitrate hypodermically.
- 9. II. Slight diarrhœa.
- 21. II. Normal. 20 mgs.
- 22. II. Normal. 20 mgs.
- 23. II. Normal. 25 mgs.
- 24. II. Normal. 25 mgs.
- 25. II. Normal. 25 mgs.
- 7. III. Normal. 75 mgs.
- 10. III. Normal 75 mgs. (No more drug given after this.)

Weight, 980 gms., salivation, sits with closed eyes, ate very little and vomited once.

11. III. Ate some meat, but sits still, weak and apathetic; salivation, tears and snuffles.
14. III. Eats fairly well, but salivates a good deal in doing so. No jaundice. Some conjunctivitis. Weight, 875 gms.
16. III. Did not eat yesterday or today. Very thin but fairly active. Weight, 820 gms.
17. III. Did not eat meat but drank some milk. Weight, 790 gms.
18. III. Found dead in the morning. Weight, 770 gms.

An examination was made at once. No subcutaneous fat was present, no jaundice and no marked congestion. One to two cc. of clear fluid in the peritoneal cavity. The pyloric end of the stomach contained a quantity of black clotted material, which was not attached to the mucous membrane. The duodenum had some clear glairy fluid, while the ileum contained black masses and the large bowel its usual contents. No erosions could be made out anywhere along the tract to account for the blood in the stomach. The gall-bladder was distended with a very light colored fluid with white masses in it. Liver weighed 35 gm.; spleen $3\frac{1}{2}$ gm., and kidneys together 14 gm. Lungs and heart seemed normal and the blood in the heart presented no abnormalities and was not lysed. Histological examination of the liver showed that organ to be in an advanced state of degeneration. The liver cells had disappeared in great part and the few to be seen stained poorly. The great bulk of the picture was occupied by blood corpuscles and their débris. Round the vessels were masses of round cells which appeared to be in process of change to connective tissue. This round cell infiltration extended also into the remains of the lobules and between the surviving liver cells. The cirrhosis had not proceeded as far as is described in cattle, but was of the same nature, and on the other hand was obviously a further development of the process seen in cats which had died from a single dose of senecifoline. In the kidney a number of small hæmorrhages were found and many of the cells of the convoluted tubules were swollen and stained badly.

A rat of 255 gms. received subcutaneously 5 mg. of senecifoline nitrate on the 1st, 9th, 11th, 18th, 21st (weight, 217 gms.)

26th (weight, 205 gms.), 29th (205 gms.), 32d (209 gms.), 35th (195 gms.), and 40th day of the experiment and was then found to weigh 168 gms., having lost about a third of its original weight. The animal was now very weak, with labored respiration, and ate very little. The coat was very rough, the hairs standing up erect and separate. It was found dead on the morning of the 41st day of the experiment. The abdominal vessels were found congested, and in the upper part of the small intestine small hæmorrhages, some of which were quite recent, were seen. The stomach seemed normal and had no hæmorrhages. The liver weighed 8 gms., did not appear congested, and macroscopically nothing abnormal was seen in it. Some hepatization and several small abscesses were found in the lungs. Microscopically the cells of the kidney tubules were found so swollen as to close the lumen entirely. The liver showed large areas of necrosis and, between these, groups of lobules in which the liver cells stained badly, while the connective tissue was increased in amount and delicate strands extended between the individual cells. Some hæmorrhages had occurred in the liver and the whole organ was much congested.

SENECIFOLIDINE NITRATE

Only a small quantity of this alkaloid was available, and this was used for experiments on rats. Its effects resembled those of senecifoline, the same changes occurring in the liver and alimentary tract. As far as could be ascertained with the small quantity at my disposal, the two alkaloids seemed to be equally toxic in the rat.

DISCUSSION AND SUMMARY

The symptoms and post mortem findings in animals poisoned with these alkaloids resemble so closely those described by Gilruth, Chase, Pethick and others, in cattle and horses, that there can be no question that the cause is the same in each and that the Pictou, Winton or Molteno disease is really more or less chronic poisoning with the Senecio alkaloids. It is true that the hepatic cirrhosis in my experiments was not so complete as seems to have

been observed in cattle, but this is sufficiently explained by the shorter duration of the intoxication in the smaller laboratory animals; and the initial stages of cirrhosis were certainly present in the two instances of chronic poisoning which I have given.

The dominating lesion present in acute and subacute poisoning is hæmorrhage which may occur in almost any organ but is constant in the liver and almost invariably present in the stomach and bowels. This is accompanied by fatty changes and necrosis of the liver cells and by great congestion of the organ. I am unable to determine which is the primary feature, whether the cell changes induce the congestion and hæmorrhage or vice versa. Very often areas of congestion alternated with areas in which the hepatic cells were swollen and stained badly or contained large globules of fat, and it appeared as if the two processes of congestion and parenchymatous change were independent of each other. These liver changes account for many of the symptoms and post mortem changes noted. Thus the thick viscous bile found in the gall-bladder may well be secondary to the hæmorrhages. A similar bile is secreted in poisoning with toluylendiamine and this has been shown by Stadelmann¹¹ to arise from the excessive bile pigment formation from the hæmolysis occurring in the liver. In senecifoline poisoning the large hæmorrhages in the liver may have the same result as the hæmolysis in toluylendiamine poisoning, the bile pigment being increased and the bile becoming correspondingly dark and viscous. In some of our cases this gave rise to jaundice of greater or less intensity and this symptom has been observed in cattle poisoned with ragwort; the yellowish color of sheep muscles after feeding on ragwort appears to be another example of this action.

The dropsy also arises in all probability from the hepatic hæmorrhages, obstructing the flow from the portal system. This was very marked in some of my experiments, and in many of them the peritoneal fluid was increased. Dropsy has also been observed in cattle poisoning.

¹¹ Joannovics and Pick: *Zeitsch. f. exp. Path. u. Ther.*, vii, p. 185.

The loss of appetite and late vomiting in my experiments are sufficiently explained by the hæmorrhages in the stomach and by the general disturbance of nutrition from the hepatic changes. Bleeding from the intestine was almost constant, in almost all cases in the duodenum, in one from the rectum. Cattle appear to pass more blood by the bowel than the laboratory animals but this appears to be merely a question of degree.

The experiments hitherto detailed were performed with the alkaloids of *Senecio latifolius*, which, as has been said, is held responsible for some of the epidemics in South Africa, and my results indicate that these alkaloids are capable of inducing the symptoms and lesions characteristic of the disease. The *Senecio jacobæa* which has been shown to be responsible for the disease in New Zealand and Canada, grows in profusion in England and Scotland, but enquiries made in various parts of the country indicate that poisoning with this plant and hepatic cirrhosis are unknown here. One hundred grammes of dried *Senecio jacobæa* (ragwort) collected in England for me in June and August were extracted with alcohol, the alcoholic solution evaporated at a low temperature and the residue taken up with water acidulated with hydrochloric acid. This was neutralized and injected hypodermically into a cat, but induced no symptoms whatever. The extract prepared in the same way from 200 gms. dried ragwort similarly gave no results when given by the stomach tube to a cat. *Senecio jacobæa* grown in Canada and dried was extracted in the same way by Professor Dunstan and sent to me for examination. A quantity corresponding to 20 gms. of the dried herb was injected into a cat hypodermically without result. If the alkaloid content of the dried herb were even as low as one per cent this dose would have contained 0.2 gm. which is certainly poisonous. These results would therefore seem to indicate that the *S. jacobæa* is devoid of the toxic properties of *S. latifolius*, whether the plant is grown in England or in Canada. This is however incompatible with the results of Gilruth and Pethick, who showed definitely that the disease in Canada and New Zealand is due to this species. The discrepancy between these results and mine may probably arise either from the plant from which my prepara-

tions were made having been collected at the wrong season, or possibly from the poisonous principle having undergone change into some inert form in the course of preparation or drying.

Senecio sylvaticus collected in Yorkshire in August proved equally inactive. *Senecio vulgaris* or common groundsel collected in England and prepared in the same way proved poisonous: a cat which received the extract from 2 gms. of the dried plant dying in ten days with symptoms resembling those arising from senecifoline, but with more marked diarrhoea. The post mortem findings were similar to those observed from senecifoline.

I hope to investigate further the toxicity of *S. jacobæa* with the hope of elucidating the curious discrepancies between my results and those of Gilruth and Pethick.

ON THE PROPERTIES OF SEVERAL SPECIES OF THE POLYPORACEAE AND OF A NEW VARIETY OF CLITOCYBE, CLITOCYBE DEALBATA SUDORI- FICA, PECK

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The properties of several species of boleti including *Boletus clintonianus* PECK, *Boletus cavipes* KALCHBRENNER, *Boletus paluster* PECK and *Boletus chrysenteron* FRIES, variety *sphagnorum* PECK, have been described previously by one of us (1) and the importance of a more extended examination of the forms included in this group indicated. Several of the tube-bearing fungi are known to possess great dietetic value, such species as *Boletus edulis*, BULLIARD, the Steinpilz of the Germans, *Boletus scaber* FRIES, and *Boletus granulatus* LINNAEUS being regarded by mycologists as important esculents. Other species such as *Boletus satanus* LENZ and *Boletus luridus* SCHAEFFER have long been looked upon as poisonous. Little is known of the toxic principles present in either of the latter forms, except for the fact that Kober (2) has found muscarine in *Boletus luridus*. The Polypores in general are large fungi containing an abundance of firm fleshy material and would be thoroughly suitable for the table were it not for the fact that certain species are apt to be infested with worms while others have a bitter taste. At the same time the tube-bearing fungi grow very commonly in America and in the majority of instances are recognised without great difficulty in view of the vast amount of study devoted to them by such authors as Peck (3), Atkinson (4) and the members of the Boston Mycological Club. The number of definitely poisonous species of boleti is not large, and since even the most toxic have such

properties as a bad taste, a violent emetic or purgative action associated with or inherent in the poisonous principles which they contain, the plants are either not eaten in sufficient quantity to cause poisoning or the active vomiting and purging which their ingestion induces prevents serious intoxication from them. Indeed the number of deaths which can be traced to the consumption of the Polypores is very small, although transient illness from their use is not so very uncommon.

Recently, through the kindness of Miss Jennie F. Conant, we have received several specimens of tube-bearing fungi from the 1910 collections of the Boston Mycological Club and take great pleasure in expressing our indebtedness to her and to the other members of the Club for the opportunity of studying this group. We have also received from Dr. C. H. Peck of Albany, N. Y., a number of plants representing a new variety of *Clitocybe dealbata*. This form when eaten by man provoked marked symptoms of poisoning and when tested upon animals has been found to possess most interesting and important properties.

The various fungi have all been examined by the methods already described, being studied for the presence of haemolysins, agglutinins, and poisons. The plant extracts have been examined chiefly with reference to their action on rabbits' blood corpuscles, while their toxicity has been determined by the subcutaneous injection of suitable amounts of the extract heated to 65° C. half an hour into both rabbits and guinea-pigs. The following species of Polypores have been investigated and their dietetic properties established as far as possible by reference to the existing literature of the subject.

BOLETUS FELLEUS *Bulliard*

This species was free from haemolysins or agglutinins. It contained no muscarine but produced in both guinea-pigs and rabbits a chronic intoxication from which the animals died in from two to three weeks. Thus a guinea-pig weighing 460 grams died in four days from 5 cc. of an extract made from 5 grams of the dried plant in 50 cc. H₂O. At autopsy a tumor mass was found in

the abdominal cavity and another guinea-pig weighing 530 grams was therefore given 5 cc. of a similar extract. This animal perished in sixteen days of a progressive cachexia. A rabbit weighing 1315 grams succumbed in twenty days to 5 cc. of the extract while a second rabbit weighing 1270 grams died from the same dosage after the lapse of thirty-one days. In both instances the animals showed a steady emaciation, their weight reaching but a little over 800 grams at death. There were no especial lesions at autopsy. *Boletus felleus* is thus definitely poisonous to both rabbits and guinea-pigs. Because of its intense and lasting bitter taste it has long been avoided by mycologists. It should probably be classed as a poisonous boletus.

BOLETUS MINIATO-OLIVACEUS Frost

An extract of this plant agglutinated rabbits' corpuscles in a dilution of one-tenth of the juice coming from 5 grams of the fungus in 50 cc. H₂O. This agglutinin was not destroyed by boiling half an hour. The extract was not haemolytic. It was poisonous to guinea-pigs one animal weighing 595 grams dying in ten days from 5 cc. and another weighing 420 grams succumbing in sixteen days to a dose of the same character. No evidence of the presence of muscarine in the plant was presented by these animals. Rabbits were not affected by the plant juice. The guinea-pigs developed a progressive emaciation but nothing characteristic could be determined at autopsy. According to McIlvaine (5) this fungus is an edible species but Collins (6) has reported the variety "*sensibilis*" as possessing poisonous properties. Other authors fail to mention the character of the species. It should probably be classed with the poisonous forms, at least until we have more knowledge of its effect when eaten.

BOLETUS CHROMAPES Frost

This species was free from haemolysin and agglutinin. No muscarine was present. The species was poisonous to guinea-pigs one animal weighing 370 grams dying in twenty-four hours

from 5 cc. of an extract of 3.2 grams of the plant in 32 cc. H₂O and a larger animal weighing 515 grams dying in fifteen days from the same amount. The latter animal showed a progressive loss in weight of over 100 grams. The plant had no poisonous action on rabbits. According to McIlvaine (7) *Boletus chromapes* is an edible species.

BOLETUS AFFINIS Peck

Contained an agglutinin active in a dilution of one-half of an extract of 5 grams in 50 cc. H₂O. This agglutinin was destroyed at 65° C. in half an hour. No haemolysin. No muscarine. No definite poisonous action upon either guinea-pigs or rabbits. Said to be edible by McIlvaine (8).

BOLETUS ORNATIPES Peck

Contained an agglutinin in a one-fortieth dilution of an extract of 5 grams in 50 cc. H₂O. This agglutinin was destroyed at 65° C. in half an hour. No haemolysin. No muscarine. No poisonous action upon rabbits or guinea pigs. McIlvaine⁹ reports it as an edible species.

BOLETUS BICOLOR Peck

Contained an agglutinin in a one-twentieth dilution of an extract of 2½ grams of the plant in 25 cc. H₂O. No haemolysin. No muscarine. No definite toxic action upon animals. Both McIlvaine (10) and Hard (11) regard the species as edible, McIlvaine stating that it is one of the very best esculents.

BOLETUS SEPARANS Peck

Contained an agglutinin in a dilution of one-quarter of an extract made from 5 grams in 50 cc. H₂O. This agglutinin resisted a temperature of 65° C. half an hour, but was destroyed by heating to 100° C. for the same length of time. No haemolysin. No muscarine. Non-toxic to rabbits and guinea-pigs. According to McIlvaine (12) and Hard (13) this is an edible plant.

BOLETUS RAVANELII Berkeley and Curtis

No haemolysin. No agglutinin. No muscarine. Non-toxic to rabbits and guinea-pigs. Dietetic qualities not reported.

BOLETUS ROXANAE Frost.

Contained an agglutinin in a dilution of one-fourth of an extract made from 4 grams in 40 cc. H₂O. This agglutinin resisted a temperature of 65° C. half an hour but was destroyed by boiling the same length of time. No haemolysin. No muscarine. Non-toxic to guinea-pigs. A rabbit weighing 1205 grams treated with 4 cc. of this extract developed a gradual loss in weight and was found dead thirty-six days later. At autopsy this animal showed no lesions like coccidiosis or pneumonia which could account for its death. It probably died of a chronic intoxication due to some peculiar substances present in the plant. No note concerning the dietetic properties of this species could be found in the literature.

STROBILOMYCES STROBILACEUS Berkeley

No haemolysin. No agglutinin. No muscarine. Non-toxic to guinea-pigs or rabbits. Edible according to Atkinson (14) McIlvaine (15) Hard (16) and other authors. McIlvaine states that it has a strong woody flavor and a faint taste of anisette or of musk.

GENERAL CONSIDERATIONS

Owing to the limited number of these observations on the various species of Polypores no very comprehensive statements can be made as to the characters of the group. One or two points of considerable interest and importance may be mentioned however. Haemolytic substances were not found in any of the species examined. Agglutinins were present in six species of boleti out of a total of nine, being found in *Boletus affinis*, *Boletus ornatipes*, *Boletus bicolor*, *Boletus miniato-olivaceus*, *Boletus roxanae* and *Boletus separans*. In two instances *Boletus affinis*

and *Boletus ornatipes* the agglutinating substances were destroyed at 65° C. in half an hour, in two species, *Boletus roxanae* and *Boletus separans* they were destroyed only by boiling, and in two cases *Boletus bicolor* and *Boletus miniato-olivaceus* they resisted even this temperature. Agglutinins were not present in *Boletus chromapes*, in *Boletus felleus* nor in *Boletus ravenelii*. *Strobilomyces strobilaceus* was likewise free from them. In but one instance *Boletus felleus* was there a definite toxicity established for both guinea-pigs and rabbits and this species is notorious for its extremely bitter taste. Several species which are known to be edible, as *Strobilomyces strobilaceus*, *Boletus separans*, *Boletus bicolor*, *Boletus affinis*, and *Boletus ornatipes* exhibited no toxicity for either guinea-pigs or rabbits. In these forms the lack of poisonous principles for animals can be correlated with their inability to produce harm when ingested by man. In one species, *Boletus ravenelii* no bad effect followed the injection of animals with the plant extract. No note has been found in the literature as to the properties of this species but in all probability it belongs to the edible fungi. In two forms *Boletus chromapes* and *Boletus miniato-olivaceus* the extracts were clearly poisonous to guinea-pigs but not to rabbits. *Boletus miniato-olivaceus* is regarded by McIlvaine as an edible species. Its action on animals and the production of definite symptoms in man by the ingestion of the closely related variety "*sensibilis*" should make us cautious about expressing a decided opinion about it, and for the present it may be looked upon as suspicious. Thus far no statements have been found of the characters of *Boletus chromapes* and a decision as to its classification may be deferred till more knowledge is available concerning its edibility. The species *Boletus roxanae* has a peculiar effect upon animals, poisoning rabbits but not guinea-pigs. Its qualities are not described in the literature and no opinion can be given in regard to its properties.

CLITOCYBE DEALBATA SUDORIFICA Peck

The species *Clitocybe dealbata* SOWERBY is considered an edible fungus, both in this country and abroad, such authors as McIlvaine (17), Hard (18) and Stevenson (19) putting a high value upon it. Specimens of a closely related plant collected by Mr. F. G. Howland at Saratoga Springs, N. Y., produced a profuse perspiration in a number of individuals. The specimens were submitted to Dr. C. H. Peck and in his case also their ingestion was followed by a sweating which began on the forehead, gradually spread over the entire body, and lasted about five hours. No other ill effects were experienced. Dr. Peck identified the fungus as a variety of *Clitocybe dealbata* giving it the varietal name *sudorifica*. His account of its botanical characters and the effect it produced when ingested appears in the New York State Museum Bulletin, No. 150 (20).

A few plants of the same lot collected by Mr. Howland were sent to us for an examination into their effect upon animals. Since but a small quantity of the fungus was available the dried plant was macerated in about twice the usual quantity of water, 0.8 grams being extracted with 16 cc. H₂O. No tests with blood corpuscles were carried out. A dose of $7\frac{1}{2}$ cc. of the extract was administered subcutaneously to a rabbit weighing 1520 grams. In a surprisingly short period of time, from three to five minutes, a profuse salivation appeared, the animal becoming weak and sick. The excessive salivation continued, accompanied by a discharge of urine and faeces, for a period of three hours. Gradually the condition of the animal improved, the salivation became less marked and eventually wore off completely. By the next morning the animal appeared as well as ever. It was kept under observation for a number of weeks and finally died of an infection. About $3\frac{1}{2}$ cc. of the same extract given a guinea-pig weighing 563 grams produced symptoms of intoxication in a few moments. The animal began to make peculiar chewing movements, lay motionless on its side and died within fifteen minutes. Another guinea-pig, 665 grams in weight injected with 4 cc. of the extract previously boiled half an hour developed a paralysis

of respiration in seven minutes, the heart continuing to beat two to three minutes after the cessation of respiratory movements. This animal showed the characteristic gasping movements of air hunger. In neither of these animals was there any unusual appearance at autopsy. In another rabbit weighing 1335 grams, 5 cc. of a similar extract produced a salivation within one minute. The saliva continued to flow from its mouth in a continuous stream for twenty-five minutes and then gradually diminished in amount. At the end of an hour the pupils of the eyes were contracted as compared with those of an untreated animal. The rabbit was likewise found to have discharged both urine and faeces. The symptoms continued till the death of the animal at the end of about two hours. The saliva from this animal was collected and 10 cc. injected subcutaneously into another rabbit. No salivation resulted in the second animal. Finally a third guinea-pig weighing 370 grams was given 1 cc. of the extract. It developed a marked conjunctival secretion within one and one-half minutes, the respiratory movements being increased in force and frequency at the same time. Within four minutes salivation appeared, the secretion from nose, mouth and eyes remaining excessive for a period of twenty minutes. These secretions now gradually diminished in intensity, and a diarrhoea developed. The animal, after a period of rapid forced respiration, died of respiratory paralysis in one and one-half hours after treatment, the heart continuing to beat for a couple of minutes, after all respiratory movements had ceased.

The pupillary constriction observable in these rabbits was brought out in a more characteristic form by the local application of the plant extract to a rabbit's eye. About four drops were instilled upon the conjunctiva of a normal animal. The pupil of this eye contracted within twenty minutes, the constriction lasting for a period of four hours.

The action of the fungus upon animals is characteristic of poisons belonging to the muscarine—pilocarpine series, the excessive salivation, conjunctival secretion, urination, diarrhoea, and stimulated then paralysed respiration being described for both bodies. Pilocarpine however does not appear to have been

found in the fungi while muscarine is a constituent of *Amanita muscaria*. *Amanita pantherina*, *Russula emetica*, and *Boletus luridus*. (See Kobert [21]). The action of this Clitocybe can thus be referred with greater confidence to muscarine than to pilocarpine. The probability of muscarine being present was rendered greater by comparing the effect of this plant extract with that of *Amanita muscaria*, various animals being treated with the two fungi side by side and a careful comparison being made of the symptoms which followed. Guinea-pigs dosed with *Amanita muscaria* exhibited the same conjunctival secretion, the same salivation, and the same diarrhoea, dying with symptoms identical with those previously observed for the Clitocybe animals. With rabbits the symptoms shown by an animal given 4 cc. of the Clitocybe extract did not materially differ from those shown by several animals with various doses of *Amanita muscaria*, the salivation and diarrhoea being the same. The Clitocybe however seemed to be much more poisonous, a rabbit weighing 1670 grams dying in less than two hours from a dose of 4 cc. of an extract made in the proportion of 1 gram to 20 cc. H₂O. Large doses, 6 cc. and 8 cc. of the *Amanita muscaria* extract made in the proportion of 1 gram to 10 cc. H₂O produced both salivation and diarrhoea in rabbits but in no instance proved fatal. This difference in the action of the two plants seemed purely quantitative and could easily be explained by assuming that the Clitocybe contained a greater amount of the poison in proportion to the size of the plant than the *Amanita*. Finally the Clitocybe extract when tested upon the exposed heart of a pithed frog showed a reaction characteristic of the muscarine-pilocarpine series. A drop of the extract diminished the frequency of the heart beat from 50–60 per minute to 5–6 per minute. A drop of a weak solution of atropine restored the activity of the heart to normal, after which several drops of the extract had but little effect, failing to reduce the frequency of the heart's action to less than 40 per minute. In another frog four drops of the extract, introduced into the ventral lymph sac, produced within ten minutes a marked diminution in the heart rate which was reduced to 5 or 6 per minute. Within twenty minutes a complete stop-

page of the heart had developed and more than one hour later the application of a drop of the atropine solution brought back the heart's action to normal within fifteen minutes. This long-continued pause is characteristic of muscarine, pilocarpine producing but a temporary cessation of the heart's activity as Harnack and Meyer have shown (22).

It is thus evident that *Clitocybe dealbata sudorifica* PECK contains a poison similar in its action to muscarine or pilocarpine, with the probability vastly in favor of the poison being muscarine. The absolute identification of this body can only be accomplished by a chemical analysis of the plant. We expect to undertake this analysis in the near future. In view of the poisonous action of this fungus upon man when ingested and upon animals as a result of subcutaneous injection it should probably be given specific rank and not be regarded as a variety of *Clitocybe dealbata* SOWERBY which apparently contains no poisonous principles acting upon man.

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- (15) McIlvaine and Macadam: One Thousand American Fungi, 1900, p. 475.
- (16) Hard: The Mushroom, edible and otherwise, 1908, p. 380.
- (17) McIlvaine and Macadam: One Thousand American Fungi, 1900, p. 93.
- (18) Hard: The Mushroom, edible and otherwise, 1908, p. 104.
- (19) Stevenson: British Fungi, 1886, vol. i, p. 78.
- (20) Peck: Bulletin of the New York State Museum, no. 150, p. 43.
- (21) Kobert: Lehrbuch der Intoxikationen, 1906, Bd. ii, 2, s. 1223, 1224.
- (22) Harnack and Meyer: Arch. f. exp. Path. u. Pharmacol., 1880, Bd. 12, s. 366.

FURTHER STUDY OF THE RELATION OF THE ADRENALS TO PANCREATIC ACTIVITY

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During the past few years considerable attention and study have been devoted to the mutual relationships which have been found to exist between various organs of the body. Those which have received most study doubtless are the pancreas, thyroid and adrenals, and the way their secretions act and react upon one another. That these organs do considerably influence one another can no longer be doubted, although in many cases the exact way in which this is done has not been explained as yet. These relationships are not confined exclusively to the so-called internal secretions of these glands but may involve also the external in the case where such exists. An interesting example of such an antagonism is the inhibition of the pancreatic secretion by adrenalin. This inhibition was first described by Benedicenti¹ in his paper entitled "The action of adrenalin upon the pancreatic secretion" which was published in 1906. In this paper Benedicenti describes the effect of subcutaneous injections of 2 and 3 mg. doses of adrenalin upon the rate of pancreatic secretion when the same was being observed in dogs with pancreatic fistulae. He found that the rate of secretion was very considerably decreased almost immediately, and in fact that it might be almost entirely stopped. The inhibitory influence lasted for a considerable time, after which the gland returned to its normal rate of secretion. If, while the secretion was inhibited by adrenalin small doses of pilocarpine were injected the latter

¹Benedicenti: Arch. Italiennes d. Biol., 1906, xcv, 1.

seemed to exert no influence in increasing the secretion, while the administration of food called forth an abundant flow.

Independently of Benedicenti this relation of adrenalin to the pancreas was described two years later by Pemberton and Sweet,² who found also that extract of the pituitary gland exerted the same influence as extract of the adrenals. In their efforts to explain this phenomenon they rejected the idea that the inhibition might be due to anemia of the gland produced by the vaso-constriction brought about by the adrenalin, basing their objections upon the following facts: (1) That the blood pressure might be raised very little by the adrenalin and yet inhibition occurred; (2) the high blood pressure produced by adrenalin might be lowered by the injection of secretin, and yet there would be no flow of juice; (3) simultaneous injections of adrenalin and secretin might not raise the blood pressure nor produce a flow; (4) old preparations of adrenalin might raise the pressure and yet produce no inhibition. They concluded therefore, that in this antagonism a certain degree of specificity existed.

As I was not entirely convinced by the arguments made to exclude the circulatory factor I made some experiments to see whether other agents which produce circulatory changes similar to those caused by adrenalin might not bring about inhibition. I was entirely successful as I found that injections of small doses of nicotine inhibited the secretion in like manner to adrenalin. After large doses of nicotine which paralyzed the ganglia along the course of the vaso-constrictor fibres I found that nicotine with its failure to affect the circulation also failed to inhibit the gland. I found also that primary doses of ergotoxin which stimulated the vaso-constrictor fibres, thus raising the general blood pressure, also produced inhibition but had no effect upon the glandular secretion when given in subsequent doses in the stage in which the constrictor endings were paralyzed. When this stage of constrictor paralysis produced by ergotoxin has come on, adrenalin can no longer cause contraction of the blood vessels, and I found that with its failure to exert this action it also failed to inhibit the pancreatic secretion.

²Pemberton and Sweet: *Arch. of Int. Med.*, 1908, 1, 628.

In addition to this evidence I found that other drugs and agents which raised the blood pressure by a constriction of the splanchnic vessels also inhibited the gland; as for instance, strophanthin and barium chloride inhibited the secretion, as did also asphyxia and stimulation of the splanchnic nerves. As I pointed out in my paper,³ I could not get so marked inhibition during asphyxia and also during splanchnic stimulation as I could from adrenalin and nicotine, and I ventured as an explanation that the latter probably caused greater anemia of the organ. The effect of splanchnic stimulation I shall discuss later in this paper.

As a result of my experiments I concluded (1) that the action of adrenalin in inhibiting the pancreas could not be considered in any sense as specific, and (2) that the inhibition was probably due to anemia of the organ, because nicotine and other drugs and agents which constrict the vessels of the glands also cause inhibition.

The suggestion of Pemberton and Sweet that there are two substances in adrenalin, one raising the blood pressure and the other inhibiting the pancreas, I could not confirm, as I found that whenever a preparation of adrenalin had not deteriorated sufficiently to prevent it having an effect upon the pressure it would also produce inhibition. Apparently these writers have come to my view of this phase of the situation, as in a later paper⁴ they say it is plain that the inhibitory properties of adrenalin are probably features of the molecule to which the blood pressure-raising principle is ascribed. They had come to this view on account of the fact that they were able to get from synthetic adrenalin effects similar to those obtained from the natural product. In the later paper referred to, Pemberton and Sweet⁵ in the main reaffirm their old views but do not offer any further evidence to support them, but do, however, make several objections to my explanation of the inhibition, or rather, they make objections to what they wrongfully consider my explanation to

³Edmunds: *Jour. Pharm. and Exp. Therap.*, 1909, i, 135.

⁴Pemberton and Sweet: *Arch. of Int. Med.*, 1910, v, 468.

⁵Pemberton and Sweet: *Arch. of Int. Med.*, 1910, v, 466.

be. On the first page of their article they say that I "ascribe the inhibition solely to the rise in pressure."

However, a careful reading of my paper shows that I did not explain the inhibition in this manner, but ascribed it to the constriction of the vessels of the pancreas upon which the increase in pressure depends. For instance, at the top of page 136⁶ I suggested that "the constriction in the abdominal vessels produced an anemia of the pancreas, and thus interfered with its function." Again on page 140, I said I used ergotoxin "as by it the vaso-constrictor action of adrenalin could be eliminated." Again, on page 145, I said that as the drugs which had "caused constriction of the abdominal vessels and a rise in blood pressure" had inhibited the pancreas, asphyxia and other agents which constrict its vessels should likewise produce inhibition. Also on page 148 I said that the inhibition was "dependent upon a certain degree of anemia of the organ"; and finally, after a further discussion I summed up my conclusions saying that adrenalin and other drugs as well as asphyxia and splanchnic stimulation cause pancreatic inhibition, which "is probably due to anemia of the organ." Reference is made, it is true, to drugs which raise the pressure, but as the rise is dependent in the cases mentioned upon vascular constriction, it is hard to see how any misunderstanding could occur, as the fundamental fact seemed to be sufficiently emphasized. Later in the paper Pemberton and Sweet do refer to the vaso-constriction in the organ, but the arguments they employ and experiments they make are directed against the theory that the inhibition is due to the general systemic rise of pressure, a view which I have never held or advocated. That this distinction is not a mere matter of words is shown by the following experiment which is planned directly to disprove this blood pressure theory of inhibition. They transfused one dog with the blood from a second, in this way raising the general blood pressure of the first dog, and yet they say they got *no* inhibition of the pancreas, the tissues being found everywhere *greatly congested*. Such an experiment as this has no bearing

⁶Edmunds: Jour. Pharm. and Exp. Therap., 1909, 1.

whatsoever upon the case, as the organs were "greatly congested" instead of being "anemic." Inhibition would not be expected.

With a clear idea then as to what my theory of the inhibition of the pancreas really is, some of the objections to it may perhaps be explained. As an aid in clearing up the difficulties connected with it, I have found of greatest assistance the plethysmographic records of the volume of the organ, a method to which I referred in my earlier paper, but one which Pemberton and Sweet have apparently not availed themselves of.

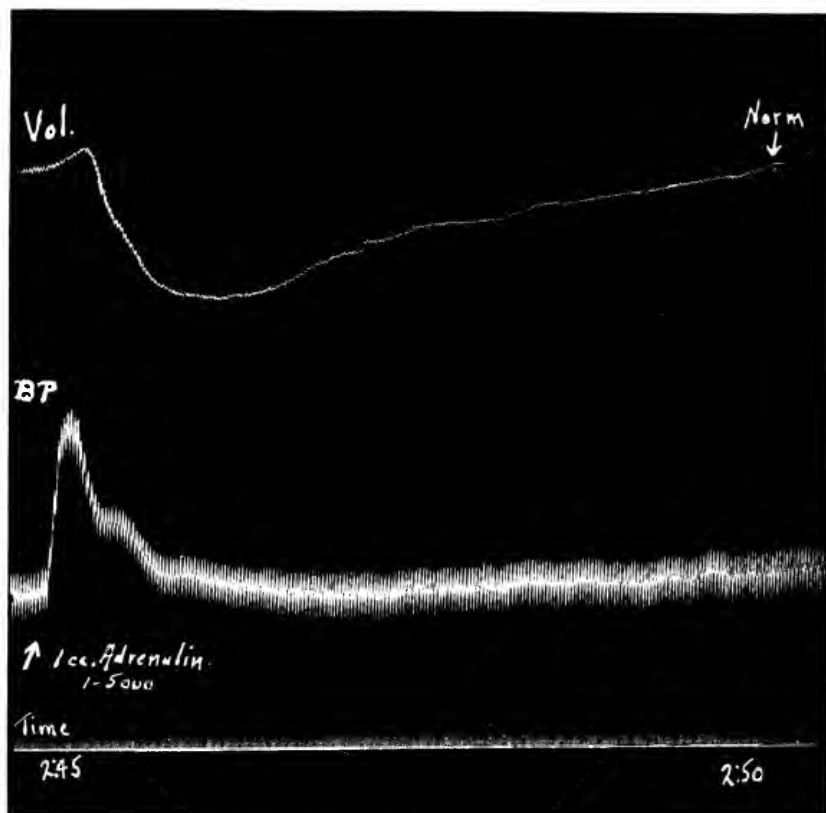
All of my experimental technic was essentially the same as described in my earlier paper. I used dogs exclusively, anesthetizing them with morphine and chloretone. A cannula inserted in the pancreatic duct was connected with a glass tube from which the drops of secretion were received on an automatic recorder.

For the plethysmographic work I constructed a suitable shaped instrument out of dental modeling wax, and closed it above with glass, rendering it air tight by means of vaseline. The instrument was connected with a piston recorder. I employed the tail of the gland alone, tying off all the vessels and freeing it from its mesenteric attachments up to about a centimeter from the opening of its duct. In this way I obtained in a medium sized dog a piece of gland about 10 cm. long.

In estimating the degree of inhibition in an individual experiment it must be remembered that in all my experiments I followed the procedure outlined in my earlier paper in keeping up a constant infusion of a dilute secretin solution, thus simulating closely the ordinary physiological stimulation of the gland and in this way securing a uniform rate of secretion. The other method of intermittent injection of secretin is very confusing, as it disturbs the blood pressure and pancreatic volume at each injection and gives no uniform rate of secretion, but one which gradually slows down making it harder to draw conclusions as regards the action of any drug. On account of its shortcomings I discarded this method very early in my work.

One objection urged against the circulatory explanation of inhibition is that the inhibition lasts very much longer than the

blood pressure rise, and presumably of course, longer than the vaso-constriction upon which the rise depends. As it is doubtless true that the blood pressure has returned to its normal level long before the secretion has been reinstated, we have to assume,



TRACING I

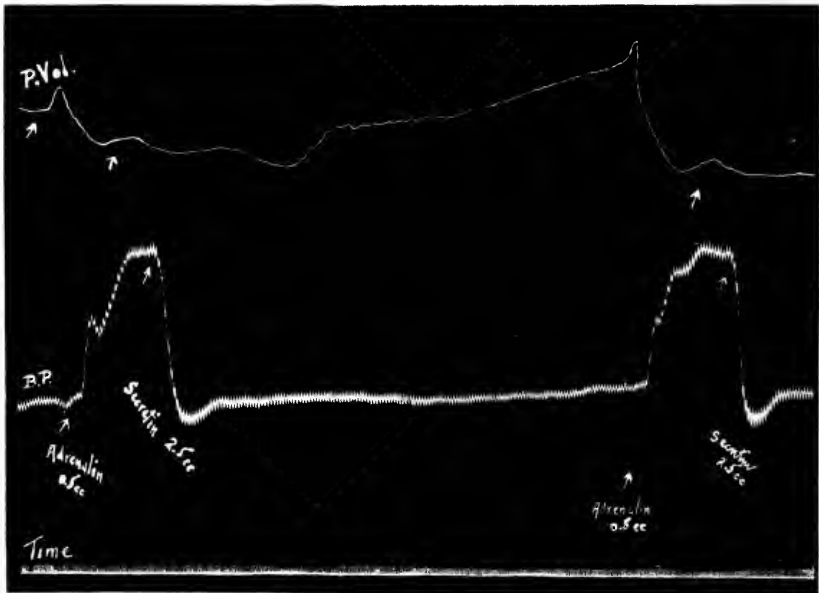
Adrenalin on the blood pressure and volume of the pancreas. The volume of the pancreas does not return to normal for some time after the blood pressure has regained its normal level.

we are told, "that the pancreas is in some way impaired at the time of the general blood pressure rise, and remains so for some time after the general system has recovered."

However, it is not necessary to assume any such injury to the pancreas as a plethysmographic tracing of the organ under the influence of adrenalin explains the prolonged inhibition. Fig. 1 shows the record of such a tracing, and it agrees in every detail with the tracings obtained by Otto May to which I referred in my earlier paper. There is an initial increase in volume of the organ, and following this a rapid diminution, which apparently does not reach its maximum until some time after the highest blood pressure has been reached. The interesting point is the length of time it takes the vessels to recover, for while the general blood pressure has returned to its normal height in from sixty to ninety seconds, the volume of the organ is not regained for a much longer time—in the tracing shown in the figure this period of time was five minutes, and in some cases the time was longer—even eight minutes. On the theory of anemia of the organ being the cause of the inhibition a sufficient explanation is at once offered by such a tracing as to why the inhibition remains after the systemic pressure has returned to its normal level. This prolonged constriction of the pancreatic vessels is not peculiar to adrenalin action as stimulation of the post-ganglionic coeliac nerve fibres has the same effect, viz. a rapid decrease in volume of the organ and a much more gradual recovery (May).

Another objection which seems to be a very serious one is urged against this theory of inhibition, viz., that when adrenalin has raised the blood pressure and inhibition is present an injection of secretin may lower the pressure to the normal level, or even below the normal, and yet we get no secretion. Here again the plethysmograph gives a complete explanation. When the blood pressure has been raised by adrenalin and the pancreatic vessels constricted and when possibly recovery is taking place with the volume of the gland gradually increasing, if an injection of secretin be now given, it may immediately lower the general blood pressure to the normal level, or even below the normal, but it does not do this by dilating the pancreatic vessels, as the pancreatic volume which had begun to increase is at once decreased by the secretin. If the injection of secretin is given while the volume of the organ is decreasing, the course of the curve is very

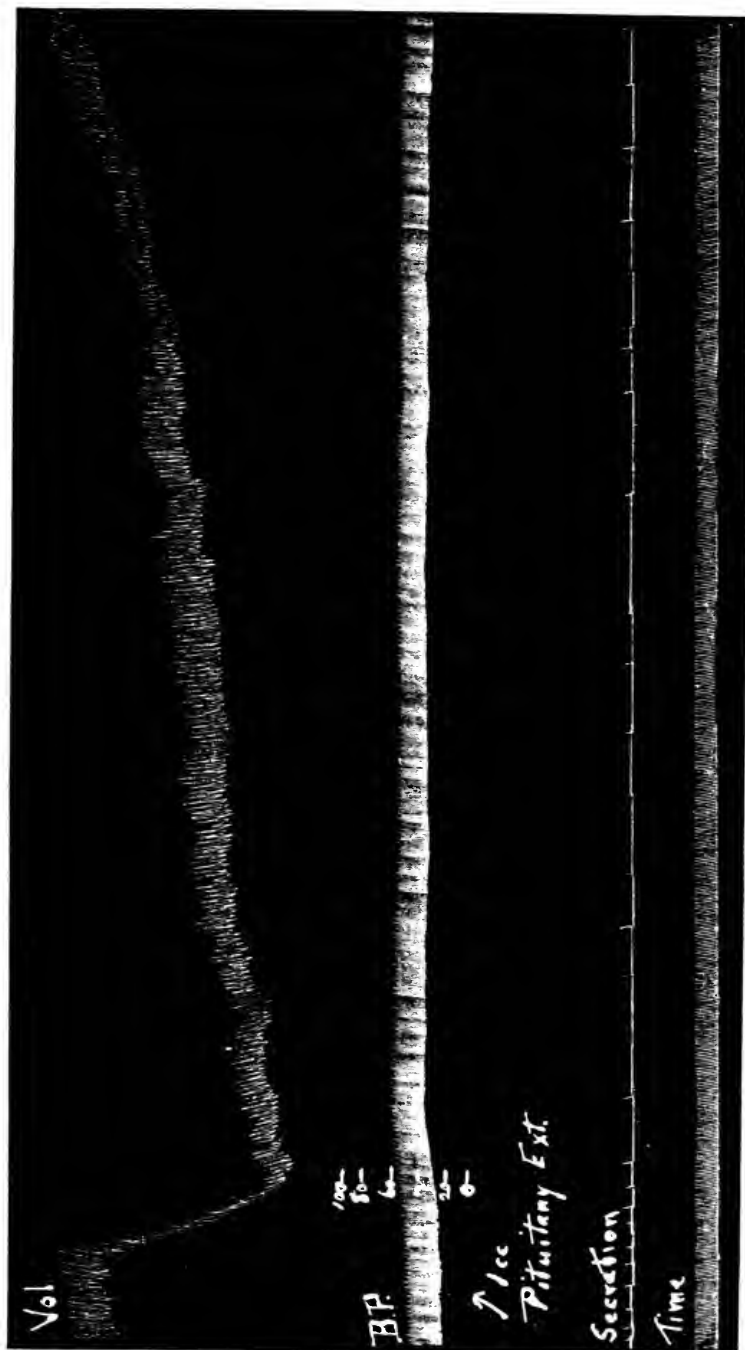
considerably changed—the fall being much more abrupt. The lowering of the general blood pressure by secretin then does not relieve the anemia from which the gland is suffering but on the other hand increases it by weakening the heart. The tracing given in fig. 2 illustrates this effect of secretin injections upon the volume of the pancreas and explains why the adrenalin-inhibited secretion is not reinstated at once by the secretin.



TRACING II

Effect of secretin injection upon pancreatic volume and blood pressure. Volume of gland lessens with fall in blood pressure produced by secretin.

Another objection which is urged is that while the extract of the pituitary gland does not raise the blood pressure to a very great extent, it yet causes marked inhibition. In fig. 3 is shown the results of an injection of 1 cc. of pituitary extract (Pituitrin, P.D. and C^o.) upon the blood pressure, the pancreatic volume and upon the rate of secretion. The record of the blood pressure taken with a von Frey tonograph shows a sustained rise in

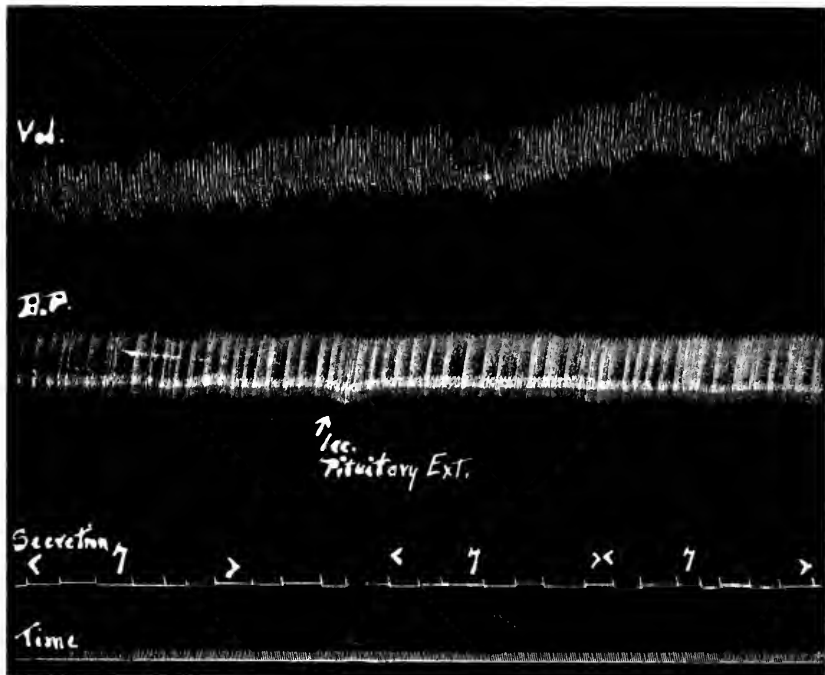


TRACING III

Effect of pituitary extract (primary injection) upon blood pressure and pancreatic volume and rate of secretion. Blood pressure record taken with von Frey tonograph. Duration of tracing was over 8 minutes.

pressure of about 10 mm., but coincident with this is a marked contraction of the pancreas which in this case does not regain its normal volume for a period of eight minutes. During this time of anemia there is very great retardation in the secretion, from the rate of nine drops per minute to two.

As pituitary extract fails to affect the blood pressure after a certain amount has been injected I continued its administration.



TRACING IV

Effect of pituitary extract (secondary injection) upon blood pressure, pancreatic volume and rate of secretion. No increase in blood pressure or decrease in volume. No change in rate of secretion produced by the injection.

In the same animal as that from which tracing in fig. 3 was obtained a second dose raised the pressure slightly, caused a slight constriction of the pancreas and decreased the rate of flow from nine drops per minute to seven. A third injection had practically

no effect upon the pressure, did not constrict the vessels of the organ, nor had it any effect upon the rate of secretion (fig. 4).

It is thus seen that pituitary extract resembles adrenalin and nicotine in causing inhibition of the gland only when it constricts its vessels (and raises the general blood pressure) but causes no inhibition when the vaso-constriction is absent.

A further point in connection with the work and the difficulty of comparing the absolute results in one series of experiments with those obtained by different workers is the question of the manner of recording the rate of secretion. In the above case cited in which pituitary extract was used the rate of secretion under normal conditions was 9 drops per minute. Now while in this instance the rate of secretion was not nearly so rapid as in some other animals, and perhaps might appear to be a "leisurely secretion," it is not fair to judge it by standards in which the secretion is recorded as it passes along a graduated tube. It would all depend upon the calibre of the tube in one case or in the other upon the size of the drops. As relative changes were the only ones of importance in this work I had not thought it necessary to try to ascertain the absolute rate of secretion until the question was raised. However, in recent experiments I have utilized a tube from which the drops are delivered at about the rate of 30 to a cubic centimeter.

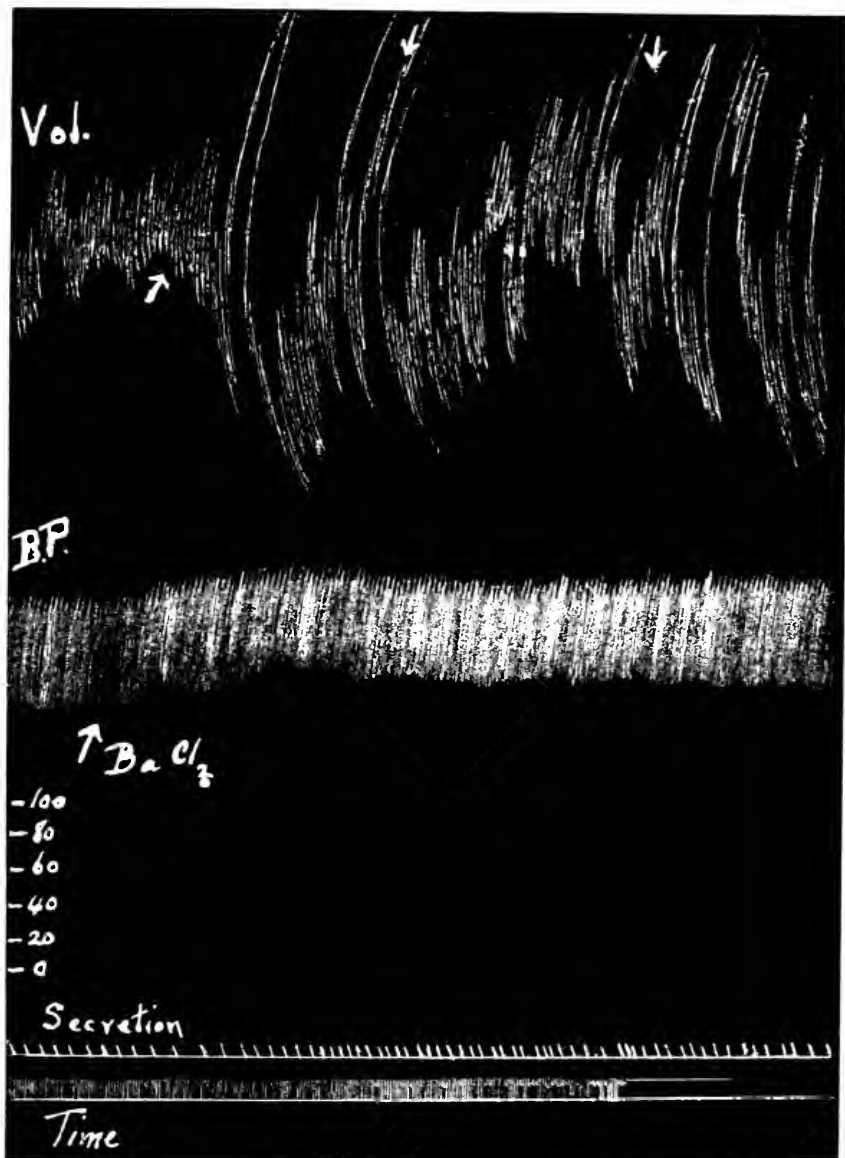
Another drug which has an interesting relation to the pancreatic secretion and the question under consideration is strychnine. As is well known, strychnine increases blood pressure by a constriction of the abdominal vessels. Accordingly I injected 1 mg. into a curarized dog whose pancreas was secreting actively. There was no change either in general blood pressure (no vaso-constriction) nor any alteration in rate of pancreatic flow. Three minutes later 2 mgs. of strychnine were injected and there followed an increase in blood pressure (vaso-constriction) of 90 mm. Hg. accompanied by total inhibition of the flow of pancreatic juice. Some time later in the experiment when the pressure had returned to the normal height a further injection of 3 mg. of strychnine produced no vaso-constriction, nor did it inhibit the pancreatic flow.

Strychnine therefore has the same effect as has pituitary extract and the other drugs mentioned in only inhibiting the pancreatic flow when it constricts the vessels.

Pilocarpine and barium chloride furnished some very interesting facts connected with the effect of the circulation upon pancreatic secretion. As I pointed out before, barium causes complete inhibition of the glandular secretion and I also found that pilocarpine exerted the same influence whether the secretion was brought about by secretin or by previous injections of pilocarpine itself. It was rather puzzling however, to find that occasionally even while the blood pressure was being maintained at an abnormally high point the secretion would begin again and in some cases be accelerated. Also in some tests with barium instead of inhibition, acceleration of the flow would be observed.

The record of the volume changes in the gland taken at the same time as the secretion rate was being recorded explained the reason for these apparently contradictory findings. Sometimes the vessels of the gland were constricted and inhibition occurred, while in others the great increase in blood pressure apparently overcame the vascular constriction and the volume of the gland was increased. The improvement in its blood supply is reflected in the accelerated flow of juice which of course may be due partially to the increased amount of secretin carried to the gland. Fig. 5 shows the volume record of the pancreas under barium chloride together with the blood pressure and rate of secretion. With the injection of barium chloride there is at first a slight decrease in volume of the gland and a momentary retardation of the secretion rate. Following this the gland increases in volume and the rate of secretion is considerably accelerated. Pilocarpine records obtained show essentially the same phenomena and so need not be reproduced. In cases where no lessening of volume was found there was no inhibition.

One point which offered a really serious difficulty to my theory of the inhibition was the fact brought out in my earlier paper that while the general blood pressure might be raised by splanchnic stimulation as well as by adrenalin, yet the drug stimulation usu-



TRACING V

Barium chloride upon blood pressure, pancreatic volume and rate of secretion. Blood pressure recorded with von Frey tonograph. Primary decrease in volume of gland, followed by great increase, with acceleration of rate of secretion.

Arrows at top of tracing show where piston recorder lever was moved down.

ally caused the greater inhibition, and I suggested as an explanation that possibly the drugs caused greater anemia of the organ. Since that time I have gone into the question further and by carefully graduating the doses of adrenalin and the strength of splanchnic stimulation I have succeeded in raising the general blood pressure to the same degree by each agent, and in such an experiment I find that exactly the same degree of inhibition is produced by each variety of stimulus. There was no difference. The details of such a protocol are given herewith. During the entire experiment a constant infusion of dilute secretin was being administered by way of the femoral vein while the adrenalin injections were given by the jugular. The splanchnic nerve was dissected out in the thorax and placed between the points of buried electrodes incased in glass T-tubing so as to permit of stimulation without disturbing the animal in any way. Artificial respiration was maintained during the experiment.

Table showing inhibition produced by adrenalin injection and by splanchnic stimulation:

STIMULATION	BLOOD PRESSURE RISE	SECRETION RATE, DROPS PER MINUTE	
		Before Stimulation	After Stimulation
	<i>mm.</i>		
Adrenalin.....	40	20	12
Splanchnic.....	40	20	12
Adrenalin.....	62	18	12
Splanchnic.....	63	14	10
Adrenalin.....	60	8	5
Splanchnic.....	60	7	4

These readings which were taken from one experiment, demonstrate very clearly that the action of adrenalin in inhibiting the pancreas is similar to that of splanchnic stimulation. The inhibition here, while it is not complete, is very distinct, as it occurs in a secretion which would be running uniformly were it not for the artificial interference. The completeness of inhibition is largely a question of dose of adrenalin; while Pemberton and

Sweet use 3 cc. of a 1-1000 solution, in my original experiments I used much smaller amounts, viz. 1 cc. of a 1-5000 solution, and in the experiment outlined above even smaller amounts were used, the dose being diminished until such amounts were given as would raise the blood pressure to an extent equal to that brought about by splanchnic stimulation.

The results obtained in the experiment given above would seem to dispose finally of any objection which could be raised against the explanation that the inhibition of the pancreas by adrenalin is due to the vaso-constriction and consequent anemia of the gland. Briefly, the evidence in favor of such a view is:

1. That after doses of ergotoxin which paralyze the vaso motor nerves, adrenalin no longer causes constriction of the vessels of the gland, nor does it inhibit it.

2. Pituitary extract will not inhibit unless it constricts the vessels of the gland.

3. Nicotine and strychnine in their primary stimulant doses constrict the vessels of the pancreas and inhibit its action. Later, after the paralytic stage has come on, neither drug lessens the blood supply to the gland, nor does either cause inhibition.

4. Other drugs such as strophanthin, pilocarpine and barium chloride which constrict the vessels of the gland cause inhibition. If, on the other hand, they increase the blood flow to the gland, as shown by increasing its volume, they have a tendency to accelerate the flow.

5. Direct electrical stimulation of the splanchnic nerve will produce precisely the same inhibition as will chemical stimulation.

The view that anemia of the pancreas will interfere with the activity of the organ is by no means new, but has been recognized for years, as I mentioned in my previous communication. Pawlow⁷ points it out, and quotes some of the older writers—for instance Bernstein, who reported in 1869 that in dogs with a permanent fistula stimulation of the central end of the vagus exerted a strong inhibition upon the normal secretion. Also Landau is quoted as finding that the stimulation of sensory nerves will

⁷Pawlow: Arch. f. Anat. u. Physiol., 1893; Physiol. Abt. Supp., Bd. 176.

produce inhibition. Pawlow himself found that if he produced anemia of the gland by stopping the heart by vagusstimulation no secretion could be obtained such as followed stimulation of the vagus when the heart was not inhibited. He also found that stimulation of the sciatic nerve by causing constriction in the splanchnic area prevented secretion when the vagus was stimulated. Gottlieb⁸ discusses rather fully the relation of the activity of the pancreas to its blood supply and points out the fact which many other writers have noted that, differing from the kidney, the pancreas is in a high degree independent of aortic pressure. He says further that the secretion seems, apart from nervous influence, dependent only upon the amount of blood which brings to the gland cells their secretion material. He also quotes an experiment of Heidenhain's as illustrating the dependence of secretion rapidity on changing width of the abdominal vessels. (In passing I may say that in one of my experiments employing secretin I encountered the same experience described by Heidenhain.) Heidenhain observed in a dog very marked periodic variations in the blood pressure. With the periodic vessel contractions a lowering of the rapidity of pancreatic secretion came in while with a lessening in vessel tone and therefore blood pressure an increase in secretion rate came in. "The connection of gland activity with vessel width was unmistakable."

Gottlieb says further that if strychnine is given to a curarized rabbit and by this means its abdominal vessels contracted, retardation of the pancreatic secretion or even complete stoppage may come in. But if one removes the vessel cramp and thereby "the anemia of the gland," by means of chloral hydrate, the secretion begins again immediately. Gottlieb also describes the effect of stimulation of the central end of the vagus. The views of Pawlow concerning the effect of anemia upon the gland activity are confirmed by Mett⁹ who brought about the same condition by splanchnic stimulation. Mett was able to call forth an insignificant secretion of pancreatic juice by splanchnic stimulation,

⁸Gottlieb: Arch. f. exp. Path. u. Pharm., 1894, xxxiii, 265.

⁹Mett: Arch. f. Anat. u. Phys., 1894; Phy. Abt., 58.

so that he says we must conclude that there are secretory fibres in these nerves, but that their activity is covered completely by the "vessel narrowing action." He thought the presence of constrictor fibres in the vagus interfered with the demonstration of secretory fibres in that nerve and so used a slow rate of stimulation to avoid stimulating the vaso-constrictor fibres and in this way was able to get a good secretion without any latent period.

Kudrewetsky¹⁰ likewise gained the impression that the presence of vaso-constrictor fibres in the splanchnics obscured any secretory action which might result from their stimulation. Accordingly he adopted the different methods employed to exclude vaso-constrictor action and was able to get a secretion from stimulation of the nerves. However, in opposition to the views of others he did not observe any stoppage of secretion with an increase in blood pressure, and so concluded that true secreto-inhibitory fibres must be present in these nerves.

Popielski has contributed extensively to various questions concerned with the activity of the pancreas. In one of his earlier papers¹¹ in discussing the inhibitory effect of the vagus he discards Mett's view concerning the action of vaso-constrictor fibres in lessening the activity of the organ as he says five to seven minutes' stimulation of the splanchnic nerve produces neither standstill nor slowing of the secretion, and so he concludes that there are secreto-inhibitory nerves present.

That stimulation of the splanchnic, either direct or indirect, will inhibit the pancreatic secretion is proved by the results obtained by many writers as I have indicated, as well as by my own experiments described herein and in the previous paper. And yet Eddins in Schäfer's *Physiology* (1900) makes the statement that stimulation of the splanchnics does not inhibit the flow, the statement apparently being given on the authority of Popielski.

Otto May,¹² employing secretin to stimulate the gland found that in the cat stimulation of the vaso-constrictor fibres caused

¹⁰Kudrewetsky: *Arch. f. Anat. u. Phy.*, 1894.; *Phys. Abt.*, 83.

¹¹Popielski: *Central. f. Phys.*, 1897, x, 405.

¹²May: *J. Phys.*, 1904, xxx, 401.

a decided slowing in the rate of secretion, but the more rapid the secretion the relatively less is the slowing produced by vaso-constriction. In the dog he occluded the aorta with a balloon, but in this case the pancreas secreted for some minutes even after the circulation was stopped, but as I pointed out earlier, there is no active constriction of the vessels in such a case, and evidently May is right in concluding that in the stagnant blood the cells of the gland are able to find material sufficient for their activity for a limited time. But this is quite different from the condition where there is active constriction of the vessels in the gland. The evidence therefore in favor of the view that anemia of the pancreas is the cause of the inhibition exerted by adrenalin would seem to be ample, whether we look to the older literature before the time of the discovery of secretin, or whether in the more recent literature and experimental work in which the gland has been stimulated by the specific hormone discovered by Bayliss and Starling.

The question whether this relation between the adrenals and pituitary on the one side and the pancreas on the other should be considered a "specific" one is not of any great importance and finally amounts to a question of words, and of interpretation of the term "specific." It would not seem as if the use of such a word in this sense was advisable. It is true that only from these two glands do we get inhibition, but that seems to be explained by the fact that up to the present time no vaso-constricting substance has been found anywhere else in the body. The vaso-constricting action certainly is not confined to the pancreas, but the influence of the adrenalin seems to be distributed as widely throughout the body as the sympathetic nervous system through which it apparently exerts its effects.

Finally when any vaso-constricting drug and even electrical stimulation of the splanchnic nerves will produce similar phenomena it would seem that we would have to stretch our commonly accepted meaning of the word "specific" in order to make it include this wide-spread action.

In a more recent paper by Sweet and Pemberton¹³ they attack

¹³Sweet and Pemberton: Arch. of Int. Med., 1910, vi, 536.

the question from the other side, viz. to study the effect on the pancreas of removing from it the control exerted by the adrenals. Accordingly one or both adrenals are removed from dogs and the rate of flow of secretion noted in the usual manner by means of a cannula inserted in the gland duct.

The removal of the adrenals causes considerable shock and the blood pressure in these animals is found to be significantly lower. In the normal control animals they found the pancreas had a tendency to secrete which apparently became more marked after a rather prolonged observation until there was a well marked flow. In the dogs with the adrenals removed they found in every case the pancreas began very shortly to secrete, and the flow increased in rapidity until in some cases the secretion was so rapid that it was difficult to record except in terms of five divisions of the cannula as units. Such a secretion obtained in a fasting animal they had only been able to duplicate with powerfully active secretin, while in one animal which had not fasted the secretion obtained exceeded at times that obtained from such secretin. The writers do not look upon the phenomena they describe as being due to removal of vaso-constriction as they found that in the control dogs the blood pressure might fall just as low as in the operated dogs and yet the rate of secretion be decidedly inferior.

It must be confessed that the task of discussing the contents of this paper is by no means easy. However, there would not seem to be any facts in it which would tend to cast doubt upon the vaso-constrictor theory of the pancreatic inhibition by adrenalin. In fact the conclusion would be otherwise. Low blood pressure *per se* doubtless does not cause secretion, but as mentioned above, many workers have found that dilatation of the vessels of the splanchnic area favor pancreatic activity. As Gottlieb says, the secretion seems dependent upon the amount of the blood coming to the gland, and this is greater when the vessels are dilated. Also the fact that some dogs have a greater flow of juice than others with equally high general blood pressure may be easily explained by individual differences in animals which seem to play a prominent part in such experiments.

All the operated dogs are said to have had lower blood pressures than the controls which fact if it was due to a vasodilation would favor secretion. The rate of secretion in the controls is said to have increased toward the end of the experiments, when there "supervenes a condition which simulates in some respects that induced earlier by the removal of the adrenals." In this connection it is interesting to note that the blood pressure of these control animals fell toward the end of the experiments.

Perhaps the greatest source of error in the experiments would lie in the failure to tie off the pylorus and prevent the gastric juice from passing into the intestine. Even if a dog has fasted for a considerable time it is very common to find acid fluid in the stomach; which fluid I have found in some of my experiments was sufficient to keep up a steady flow of pancreatic juice, while on the other hand, if the pylorus is tied off, the secretion of the pancreas stops in a very short time.

In the experiments in which the adrenals were removed it required very considerable manipulation and handling of the abdominal organs. This would certainly favor an overflow of gastric juice into the intestine and thus secretin formation and pancreatic activity. Such a series of experiments are under quite different conditions from those in which the only manipulation necessary was to insert a cannula in the duct, when the stomach does not have to be touched, and the latter experiments cannot be considered as "controls" for the former.

This supposition would seem to be borne out by the difference in reaction between a fasting and a non-fasting dog. The latter would naturally have more gastric juice, and therefore more to be forced into the intestine. As secretin would be formed in either case it is hard to see how the reaction in a non-fasting dog could be greater than would result from the injection of "powerfully active secretin." Finally, they summarize their findings as follows: A dog with adrenals intact gives practically no flow or relatively little; with one adrenal removed there is a tendency to give some flow, although often none; with both adrenals removed there is a "marked flow." On the "specific" inhibitory theory it is rather hard to explain their findings because, as the

writers have already demonstrated, the pituitary gland not having been removed in these experiments it would still be exerting its specific inhibitory influence. On the circulatory theory the results are easy of explanation.

In those animals with the adrenals removed in which there was such an active secretion, exceeding in rate even that obtained by powerfully active secretin, it would have been very interesting to have removed the pituitary gland also, and to have seen whether the rate would have been still further increased.

They report also that internal hemorrhage prevented or modified the "terminal adrenal flow." This it would be expected to do, for while the systemic blood pressure would be low there would be anemia of the organ due to the loss of blood and possibly a compensatory vaso-constriction.

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